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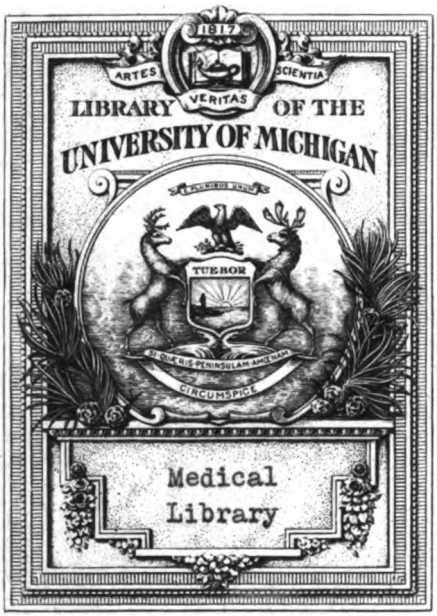

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TRANSACTIONS OF THE

SECTION ON

Pathology and Physiology

of the
American Medical Association
at the Sixty-Fifth Annual
Session, held at Atlantic City,
N. J., June 23 to 26, 1914

AMERICAN MEDICAL ASSOCIATION PRESS
CHICAGO: NINETEEN HUNDRED AND FOURTEEN

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LIST OF OFFICERS

Those named below have served as officers in this section. This branch of the practice of medicine has been included with other subjects in the earlier years, as will be seen by the headings which denote the different section titles. The names of the officers have been taken from the published records, which are deficient in some cases. It will be appreciated if any additional data are brought to the attention of the Secretary of the American Medical Association.

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PROCEEDINGS OF THE SECTION

Tuesday, June 23—Afternoon

The meeting was called to order at 2:15 p. m. by the chairman, Dr. William Ophüls, San Francisco.

Dr. Ophüls then read his chairman's address, entitled "Anatomic Structure and Function." No discussion.

"Dr. J. C. Friedman, Chicago, read a paper by himself and Dr. W. W. Hamburger, Chicago, entitled "Experimental Chronic Gastric Ulcer." Discussed by Prof. A. J. Carlson, Chicago, and Drs. E. C. Rosenow and J. C. Friedman, Chicago.

Dr. J. W. Draper, New York, presented "Studies on Duodenal Obstruction." Discussed by Dr. J. C. Bloodgood, Baltimore.

Dr. J. T. Case, Battle Creek, Mich., read a paper entitled "Roentgenographic Studies of the Function of the Ileocolic Valve." Discussed by Drs. L. G. Cole, New York; Walter B. Cannon, Boston; Prof. A. J. Carlson, Chicago; A. C. Eustis, New Orleans; Seymour Basch, New York; E. C. Rosenow, Chicago; G. E. Pfahler, Philadelphia, and Thomas A. Groover, Washington, D. C.

Dr. F. M. Allen, New York, read a paper on "Studies in Experimental Diabetes." Discussed by Prof. A. J. Carlson, Chicago, and Dr. A. C. Eustis, New Orleans.

Prof. A. J. Carlson, Chicago, presented a paper entitled "Blood Transfusion in Pancreatic Diabetes." No discussion.

Dr. L. F. Watson, Oklahoma City, Okla., read a paper on "Urea and Quinin Injection in Goiter." No discussion.

Wednesday, June 24—Morning

A joint meeting was held with the Section on Practice of Medicine.

The following papers were read as a Symposium on Nutrition:

Prof. L. B. Mendel, New Haven, Conn.: "The Specificity of the Nutritients."

Prof. Otto Folin, Boston: "The Intermediary Metabolism."

Prof. Graham Lusk, New York: "The Specific Dynamic Action of Food."

Dr. Eugene F. Du Bois, New York: "The Total Energy Requirements in Disease as Determined by Calorimetric Observations."

These four papers were discussed by Drs. Victor C. Vaughan, Ann Arbor, Mich.; S. J. Meltzer, New York; F. M.

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Pottenger, Monrovia, Cal.; E. P. Joslin, Boston; A. D. Eustis, New Orleans; George Douglas Head, Minneapolis; A. J. Carlson, Chicago; Graham Lusk, New York, and Eugene F. Du Bois, New York.

Wednesday, June 24—Afternoon

A joint meeting was held with the Section on Obstetrics, Gynecology and Abdominal Surgery, and the Section on Pharmacology and Therapeutics.

The following papers were read as a Symposium on Specific and Protective Ferments:

Prof. Emil Abderhalden, Halle, Germany: "The Present Status of the Investigation." (In the absence of the author the paper was read by Prof. A. J. Carlson, Chicago.)

Dr. Victor Vaughan, Ann Arbor: "Parenteral Protein Digestion."

Dr. Chester M. Echols, Milwaukee: "Limitations of the Dialysis Method as a Practical Test for Pregnancy."

Dr. Henry Schwarz, St. Louis: "The Value of Abderhalden's Biologic Reaction to the Obstetrician and Gynecologist."

These four papers were discussed by Drs. Herman J. Boldt, New York; Charles S. Bacon, Chicago; Philip Williams, Philadelphia; F. H. Falls, Chicago; Charles Goodman, New York; Michael G. Wohl, Philadelphia; Clarence F. Ball, Rutland, Vt.; O. Lowry, Newark, N. J.; A. J. Carlson, Chicago, and Henry Schwarz, St. Louis.

Thursday, June 25—Morning

Meeting called to order by the Chairman, Dr. William Ophüls, San Francisco, at 9 a. m.

The following officers were nominated by the Nominating Committee and unanimously elected, the Secretary being instructed to cast the ballot: Chairman, Prof. A. J. Carlson, Chicago; Vice-Chairman, Dr. L. B. Wilson, Rochester, Minn.; Secretary, Dr. Frederick P. Gay, Berkeley, Cal.

Dr. L. D. Bulkley, New York, read a paper entitled "Relation of Diet to Cancer." Discussed by Prof. A. J. Carlson, Chicago, and by Dr. L. D. Bulkley.

Dr. Maude Slye, Chicago, presented a paper on "The Influence of Heredity on the Incidence of Cancer in Mice." Discussed by Drs. F. M. Baeslack, Detroit; L. D. Bulkley, New York; G. Luden, Rochester, Minn.; H. C. Schmeisser, Baltimore, and Prof. A. J. Carlson, Chicago.

Dr. C. F. Ball, Rutland, Vt., read a paper on "Abderhalden's Test in the Diagnosis of Cancer."

Dr. F. H. Falls, Chicago, read a paper on "A Study of the Ferment Activity of the Blood-Serum During Pregnancy and Under Normal Pathologic Conditions by the Abderhalden Method."

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These two papers were discussed by Drs. Charles Goodman, New York, who presented a lantern-slide demonstration; M. G. Wohl, Philadelphia; C. P. McCord, Detroit; C. F. Ball, Rutland, Vt.; F. H. Falls, Chicago, and Prof. A. J. Carlson, Chicago.

Dr. D. S. D. Jessep, New York, presented "Adenomyoma of the Rectovaginal Septum." No discussion.

Dr. B. F. McGrath, Rochester, Minn., read a paper on "Carcinoma of the Prostate." No discussion.

Dr. C. P. McCord, Detroit, read a paper on "The Pineal Gland in Relation to Somatic, Sexual and Mental Development." No discussion.

Thursday, June 25—Afternoon

Dr. L. F. Bishop, New York, read a paper on "The Pathologic Physiology of Arteriosclerosis." Discussed by Drs. W. M. L. Coplin, Philadelphia, W. Ophüls, San Francisco, and L. F. Bishop, New York.

Dr. G. B. Webb, Colorado Springs, read a paper by himself and G. B. Gilbert on "Immunity in Tuberculosis." Discussed by Drs. William Ophüls, San Francisco, and G. B. Webb, Colorado Springs.

Dr. R. B. H. Gradwohl, St. Louis, read a paper on "The Hecht-Weinberg Complement-Fixation Test as a Control Over the Wassermann." No discussion.

Dr. W. McKim Marriott, St. Louis, read a paper on "The Blood in Acidosis from the Quantitative Point of View." Discussed by Prof. A. J. Carlson, Chicago, and Dr. W. McKim Marriott, St. Louis.

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ANATOMIC STRUCTURE AND FUNCTION *

W. OPHÜLS, M.D.
SAN FRANCISCO

Work well done along any line of human endeavor is a satisfaction and a reward in itself. Still it behooves us to pause once in a while in the press of our labors in order to ascertain whether the direction which we are following is the correct one, whether there is promise of progress commensurate to the effort employed or whether a shifting of the point of attack or a change of the means employed may not give better result. This seems to apply with special force to pathology at the present moment, when we hear such warnings as that addressed to us by Yandell Henderson, who, in 1911, stated before this section that "a man trained by autopsy and microscope is inevitably a morphologist. Just in proportion as he is fitted to deal with morphologic processes he is unfitted to handle and expound functions of a physical nature—unless, of course, he happens to be an all-around genius," or when we hear the repeated admonition of such men as Meltzer to pay less attention to morphologic problems and more to functional studies. If Henderson's assertion is at all correct, present conditions are truly deplorable, because many men have been and still are being trained by necropsy and microscope and if this study unfits them to understand function in its proper light, the sooner all efforts in this direction cease the better, because I cannot conceive of any object in morphologic research except as a help to the clearer understanding of function.

* Chairman's Address.
* From the Pathological Laboratory of the Stanford University Medical School.

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The question of the relation of structure to function is the great biologic problem, if we take structure in its broadest sense as including the structure of the chemical substances which make up the living protoplasm. In the last resort our interpretation of the relation of structure to function will rest on our conception of the relation of force to matter, and, as I believe we are all agreed that these two expressions designate merely two human ways of looking at the same thing, we might come to the conclusion that the whole discussion is idle and entirely futile. I do believe that this identity, if I may say so, of structure and function is sometimes lost sight of to the detriment of scientific reasoning, although nature, if we only look at it with open eyes, constantly reminds us of this fact, no more commonly perhaps than in the field of pathology. Looking at the question from this broadest point of view it does not require much of an argument, for instance, to convince us that function determines structure just as in its turn structure determines, delimits—that is, places definite limits on function. Still, what we grant theoretically, must be worked out practically. It is with the practical imperfection of our knowledge that we are concerned and with the question of how best to improve on it.

Any student of medical history knows how much our knowledge of physiology has advanced with the gradual progress in gross anatomy and how much more many of the intricate problems were clarified by the knowledge of the finer structure of the tissues as revealed by the microscope. Our fundamental conceptions with regard to the cell as the living unit, with regard to cell life and cell activity, could not have been arrived at without the help of the microscope, and on this same solid basis of gross anatomic and histologic knowledge rests modern physiology, and with that pathology.

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It is only natural that the splendid advances made by the introduction of the microscope aroused excessive expectations in the minds of some as to what might be accomplished by the use of this instrument. We certainly know now that it has its great limitations so far as power of vision and its applicability to living tissue is concerned; nevertheless, the insight which we have gained by means of it into physiologic and pathologic processes is truly remarkable.

If I may speak from the point of view of a pathologist, the condition of affairs at present seems to be something like this:

There exists quite a large and constantly growing territory in this science in which our knowledge of the gross and microscopic anatomy furnishes a very adequate explanation of the functional disturbances which we observe during life. This is especially true if we include parasitology, gross and microscopic, although here also the limit of usefulness of the microscope is being reached as shown by the experiments with filtrable virus.

It must be emphasized, however, that structural change does not necessarily mean change in function. I believe that in this regard the importance of minute histologic lesions in the tissue has been greatly exaggerated. We must constantly bear in mind, especially in the important glandular organs, the existence of a large amount of reserve tissue which must be eliminated before the function of the organ as a whole will be affected. We also have to take into account the remarkable power of regeneration and of compensatory hypertrophy which these organs possess. Even when we give due consideration to these facts, however, it is often astonishing how well an organ has functionated during life in spite of the finding at necropsy of pretty definite and fairly diffuse lesions.

In fact, in some cases, the question arises whether the lesions which we find associated with disturbance

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of function are really the cause of the disturbance or merely concomitant. The mere fact that the development of clearly manifest lesions usually consumes considerable time (usually twenty-four hours and often much more) is very suggestive in this regard. If the lesions are to be the cause of the disturbance in function which we observe, they must precede the disturbance, but should not follow it. If, for instance, we produce a disturbance of the renal function by means of some renal irritant which manifests itself by albuminuria we observe the disturbances of function, if the irritation is sufficiently strong, within less than one hour, at which time even the most careful histologic study fails to reveal any change, whereas well-marked anatomic lesions develop as a rule in the course of the next twenty-four or thirty-six hours. Such lesions cannot very well be the cause of the disturbance in function; still they do indicate that by the insult the cells were injured in such a way as to lead eventually to a change in their structure. In other words, what we see is not the cause of the disturbance in function but the final result of a cell injury the actual nature of which is not demonstrable by the means at present at our disposal. This appears to me to be the reason why so many of the degenerative lesions resemble autolytic changes which take place in the dead tissue. Still, they are valuable, because after a while at least they do show us that the cells have been injured. I believe, however, that it is worth while to emphasize this fact that sometimes the lesions are secondary to a primary insult not associated with demonstrable anatomic changes because in the hurry of our teaching or in the abbreviated parlance of even scientific reports it is often lost sight of.

It has been said by some that further efforts in the refinement of our technic in this direction are apparently hopeless and a mere waste of time, and there may be some grain of truth in this statement. Still, I believe it is hazardous to prejudge a case of this

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kind and it would seem to be better policy to encourage men who have a natural bent along these lines, because, after all, the better the fundamental facts relating to the structural minutiae of the cells are worked out, the more secure the structure of our scientific edifice will be.

On the other hand, I know, from personal experience that there are large and important fields in pathologic anatomy which need much new work before they are anywhere near their possible completion. From this point of view, in many cases, the surface seems hardly to have been scratched. The common types have been fairly well established, but the more intricate problems, especially again in relation to the functional disturbances, have been hardly worked up at all. When we descend from the textbook picture to the individual case and to a careful scrutiny of its individual problems, we find no end of stimulation to investigation and research, even along anatomic lines.

In our study of function we cannot of course rest entirely on an anatomic basis, because evidently many problems connected with this subject are not open to anatomic investigation. The structural changes which underlie them are so minute that they cannot be approached by means of the scalpel or the microscope. Recently, for instance, studies have been carried on in our laboratory in regard to the severe disturbances in nitrogen metabolism which follow anaphylactic shock. They do not seem to be associated with any discernible lesions in any of the important organs, in spite of the fact that they persist for a considerable period of time and eventually cause the death of the animals. This is by no means an isolated example even if we exclude from consideration the central nervous system, the mysteries of which, in spite of great advances in our knowledge, are still so baffling.

In many of these problems undoubtedly chemistry or physical chemistry will step in and furnish us new

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clews. Judging from the material which has already accumulated along these lines the prospect for further important developments seems to be unusually bright. Still there are intrinsic difficulties in method which it will be difficult to overcome. As Meltzer has pointed out, chemical research labors under the same disadvantage as pathologic anatomy in dealing largely with dead material. There is also the distinct disadvantage of being confined so far to rather gross procedures which do not permit of a utilization of our knowledge of the finer histologic detail of the organs studied.

Microchemistry, that is, the application of chemical methods to histologic sections, has bridged over this gap in some instances and may do so in more, but, unfortunately, characteristic chemical reactions are to be obtained from bodies only when they are more or less dissociated, and the more intimately they are bound up with the living protoplasm, the less easily they are detected by chemical means without resorting to violent interference.

On account of these difficulties in the way of a direct application of exact chemical and physicochemical methods, a large body of hypotheses has developed which is based on our present knowledge in organic chemistry, but which, so far, is beyond the reach of actual demonstration. I need only to allude to our chemical conceptions with regard to enzymes, toxins and the various immunity reactions in order to bring out this point clearly. It is to be hoped that a refinement of our present methods will permit us eventually to develop this knowledge to a satisfactory conclusion, and if we do succeed, our ideas with regard to the relation of chemical structure to function will necessarily be advanced immeasurably.

Progress along any of the lines mentioned—pathologic anatomy, parasitology, biologic chemistry and biologic physics—is absolutely impossible without experimental work. In fact, the animal experiment

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is the touchstone to any new idea which may develop in the field of biology, including medicine. The final proof of any contention in the biologic sciences rests in the animal experiment. On the other hand, the experiment needs the control of the other methods in order to make quite certain that the experimental results are applicable to the case in question.

The experimental study of function itself is naturally of the greatest importance, quite immaterial whether we understand the underlying factors or not. For practical purposes the value of such experimental researches may be so great that it may well be questioned whether some of the energy now directed to the elucidation of more obscure general problems could not with advantage be diverted along these new and fruitful channels. If we know the modifications in function arising from physical or chemical changes in the environment of the functionating cell, we at once obtain means of controlling such function more or less, and what this means to the physician who has to deal with manifestations of disturbed function in his patients needs no amplification. In this way he frequently may obtain the desired effect in spite of the fact that the ultimate mechanism of the action is unknown, and a complete knowledge of the same possibly unattainable.

Such studies of function under carefully selected and well-controlled experimental conditions may also permit of very interesting conclusions with regard to the mechanism involved. Our knowledge of the chemical control of glandular function has been obtained in this way. There is also no question that some serious errors arising from a merely anatomic study of the parts involved have been corrected by this method, but after all it is a method with its own advantages and its own palpable limitations like all others.

In conclusion, then, I wish to emphasize that our goal of a thorough understanding of the biologic

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processes in health and disease cannot be reached by one method alone. Men with varying inclinations and varying training have to cooperate in order to make progress, and the more intricate our problems grow the more the necessity of such cooperation becomes evident.

It is of course an impossibility for any man to be a master in all branches of biologic research, but a fair degree of familiarity with all is necessary, to produce, if I may borrow a German expression, a friendly understanding, to avoid misconceptions and the development of that narrowness of view to which I imagine Professor Henderson wished to refer. I believe that laboratories in pathology, just like those in physiology, pharmacology, hygiene and clinical medicine, should be ready at any time to take up a problem from whichever point of view appears to be the most hopeful, with whatever methods seem to be indicated, and, if necessary, from as many points of view and with as many variations in method as seem desirable.

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EXPERIMENTAL CHRONIC GASTRIC ULCER

A SECOND CONTRIBUTION TO THE EXPERIMENTAL PATHOLOGY OF THE STOMACH*

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AND
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It is comparatively easy to produce experimentally acute ulcers in animals, and as far as gross and microscopic examinations go they resemble the same conditions in man. Common to all ulcers so produced, however, is the fact that they heal rapidly, healing being complete in the great majority of cases in from two to three weeks. In the human chronic ulcer, however, hyperacidity is present in at least half the cases, and it seemed to us, then, that if we added this factor to experimental acute ulcer, chronicity might be induced. Furthermore, it is a well-known clinical fact that stomachs with partial pyloric obstruction generally show hyperacidity, or, what is probably synonymous, hypersecretion; consequently we might induce hyperacidity experimentally by partially obstructing the pylorus and later in the same animals cause acute ulcers. To this end we tied a heavy silk ligature around the pylorus of dogs, just tight enough to produce moderate tension and marked peristalsis and antiperistalsis. We found that if just the right amount of tension is used, such stomachs will show marked dilatation and hypertrophy in from two to three weeks. If the ligature is tied more loosely, no effect is produced; if it is tied more tightly, complete pyloric

* From the Nelson Morris Memorial Institute for Medical Research.

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obstruction results, with sudden exitus in from forty-eight to seventy-two hours, and symptoms at times resembling tetany. In animals successfully operated on, test meals from two to three weeks later show both stasis and hyperacidity.[1]

The test meals employed consisted of 300 c.c. milk and 100 gm. chopped beef, the latter fed, the former introduced through a stomach-tube. Fifty minutes later 1/20 grain apomorphin was given hypodermically, and the vomitus caught in a vessel, measured and examined in the usual way. As far as possible, the test meals were given the dogs before and after each operative procedure.

The next step after producing a partial obstruction was to produce acute ulcers in these stomachs. This is comparatively easy, any number of methods having been previously successfully employed, and as far as the present work is concerned, any one of them might have been used. In general, the methods used have paralleled the clinical theories as to the etiology of ulcer, and may be divided broadly into (*a*) infectious, (*b*) toxic, both local and general, and (*c*) mechanical.

By infectious methods (*a*), acute ulcers have been produced by injecting various bacteria intravenously, such as streptococci, staphylococci, the *Bacillus coli,* pneumococci, etc., and by feeding bacteria. The toxic methods (*b*) have been either general, such as those observed by injecting diphtheria antitoxin, or local by injecting silver nitrate, epinephrin, liquor formaldehyd, nicotin, etc., into the walls of the stomach. The mechanical methods (*c*) include excising pieces of mucosa, tying off gastric arteries, and injecting various emulsions into gastric vessels. We selected the injection of silver nitrate into the submucosa as the surest method, using from 0.5 to 1 c.c. of a 5 per cent. solution. Each animal was injected in two or three places at the final operation, one injection being made

1. Dr. Hamburger will report elsewhere in detail on these and allied phases of our work.

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near the pylorus, one near the middle of the lesser curvature, and one in the fundus.

These injections produce necrosis of the wall, and in from forty-eight to seventy-two hours the slough separates, being rapidly digested, and leaving ulcerated areas of varying depths and breadths, sometimes with smooth edges, as in Figure 1 *A*, sometimes with ragged edges, as in Figure 1 *B*. Perforations are frequent, hemorrhages much more seldom. Such ulcers, if produced in normal stomachs, heal in from two to three weeks. To return to the results of partial pyloric obstruction: These are shown in Tables 1 and 2, the first including cases in which post-mortems were done three weeks or more after the second operation, and unhealed ulcers found, the second, cases in which they had completely healed.

Of sixteen animals successfully operated on, the average total acidity before pyloric obstruction was 34, the average of all our animals being 30, the average, from three to four weeks after such obstruction, 50. Such increase, however, was not necessarily permanent, as in some cases, four weeks or so later, the acidity had dropped again, as in Case 51, Table 2, in which it dropped from 54 to 30, and in Case 42, Table 2, from 55 to 36. Another point is the total quantity of stomach-contents removed. This quantity varied in normal animals from 100 to 350 c.c., averaging about 200 c.c. After successful obstruction operations, the amount rose to various heights up to 1,000 c.c., and in general it can be seen from the tables that hyperacidity is present only in those cases in which the total contents are excessive, and, furthermore, that in some cases in which the total quantity of stomach-contents rose, the acidity rose with it. So in Case 41, Table 2, in which the total contents rose from 200 to 425 c.c., the acidity rising from 37 to 52 during this period, and in the cases mentioned above, Cases 51 and 42, Table 2, in which the acidity dropped to normal, the total quantities dropped also. The coincident variations of

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these two factors point to some relation between them, probably an identity. As stated at the outset, then, hyperacidity is probably simply a hypersecretion, and in many cases is dependent on impaired motility. We hope in the near future to work at this problem.

To come now to the main problem, a comparison of these two tables shows the following facts:

In every case of Table 1 there was marked impairment of motility, as measured by the total quantity of stomach-contents recovered after test meals, and by the condition of the stomach found postmortem as to hypertrophy and dilatation. In each case a notation was made at necropsy, "greatly dilated," or at least "greatly hypertrophied," while only one case in seven of Table 2 (namely, of healed ulcers), was greatly dilated. The acidities varied, as stated above, and again it was in those cases in which the motility was most impaired, as measured by total stomach-contents and post-mortem dilatation, and the hyperacidity most pronounced, that the chronicity of the ulcer was most marked. So in Cases 48, 37, 22, 5, 55 and 50 (Table 1), from which our best specimens were obtained, the dilatation was very marked and the acidity high.

The types of ulcers which we have so produced can be divided into three classes: First, those which are apparently acute ulcers, in which healing is simply delayed. They have a puckered appearance, as though scar formation were slowly progressing (Fig. 2). These are similar to the ones described by Bolton,[1] who partially obstructed the pylorus in cats and considered this the only type so obtainable. Second, typical chronic ulcers, probably stationary, with rounded, overhanging edges and smooth fibrous bases (Fig. 3). Third, progressive chronic ulcers, as in Figure 4, in which the induration is not marked, but the evidences of autodigestion are evident, as indicated by the terracing at one end and the deep undermining at the

1. Bolton: Further Observations on the Pathology of Gastric Ulcer, Proc. Roy. Soc., 1909-1910, lxxxii, B.

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TABLE 1.—CHRONIC ULCERS

Case No.	Duration of Ulcer, Weeks	Stomach Contents				Condition of Stomach at Necropsy	Ulcers
		Acidity after First Operation	Acidity after Second Operation	Quantity after First Operation, c.c.	Quantity after Second Operation, c.c.		
48	5½	26	50	450	550	Moderate dilatation, great hypertrophy.	Two chronic pyloric ulcers. Two spontaneous duodenal ulcers.
35	9½	..	38	...	150	Small, slight hypertrophy.	Two small healing ulcers.
36	7½	..	26	...	350	Slight dilatation.	Small chronic pyloric ulcer.
37	8	..	50	...	350	Great dilatation.	Deep pyloric ulcer, 10 by 12 mm.
43	6½	25	36	400	200	Slight dilatation, great hypertrophy.	Chronic pyloric ulcer, 6 by 15 mm.
22	3	..	..	...	...	Great dilatation.	Chronic pyloric ulcer, 2 by 1 cm.
5	6	23	..	...	...	Great dilatation.	Healing ulcer, lesser curvature, 4 by 4 mm. Chronic pyloric ulcer.
55	7	56	42	950	1,050	Great dilatation.	Small chronic pyloric ulcer, .05 by 1 cm.
50	4½	75	64	300	250	Great dilatation and hypertrophy.	Superficial pyloric ulcer, 1 by 1 cm.

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TABLE 2.—HEALED ULCERS

Case No.	Duration of Ulcer, Weeks	Stomach Contents				Condition of Stomach at Necropsy	Ulcers
		Acidity after First Operation	Acidity after Second Operation	Quantity after First Operation, c.c.	Quantity after Second Operation, c.c.		
38	7	26	36	...	550	Moderate dilatation.	None.
41	7½	37	52	200	425	Small.	None.
51	4½	54	30	350	200	Small.	None.
39	?	..	62	...	800	Great dilatation.	None.
42	?	55	36	250	325	Slight dilatation.	None.
26	2	..	..	...	...	Small.	One acute pyloric ulcer. One acute ulcer lesser curvature.
28	3	..	..	...	...	Small.	Two healing ulcers.

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other. No typical callous ulcers were found, the nearest approach being the one shown in Figure 5, in which the induration is limited to the base, which consists of excess of granulation tissue.

In two cases spontaneous duodenal ulcers were found at necropsy, just beyond the pylorus. In one case the thickened mucosa showed superficial ulceration. In the second case, Case 48, two typical round ulcers were present, one of which had perforated and caused peritonitis. These cases will be reported on later.

It will be remembered that each animal was injected with silver nitrate at the second operation in two or three different places, near the pylorus, lesser curvature and fundus. By reference to Table 1, it will be seen that in the majority of cases the pyloric ulcer alone remained unhealed, or in the cases in which two remained, the one near the pylorus was much larger in every way than the other. This is quite in accord with statistics in man, for instance, Fenwick's who states that acute ulcers are multiple in 50 per cent. of the cases, and situated irregularly throughout the stomach, while chronic ulcers are single in 80 per cent. of the cases, and situated near the pylorus in 75 per cent.

The simplest interpretation of these facts would be as follows: Acute ulcers tend to remain unhealed when exposed to the action of a very active gastric juice for an abnormally long period, and possibly the delay in healing is greater if the food and gastric juice are ground against the ulcers with unusual violence from hyperperistalsis. Consequently, at least three factors are necessary for the production of chronic ulcers in animals: first, a local destruction of the mucosa; second, an active or overactive gastric juice and third, prolonged or vigorous contact of the two (hyperperistalsis). This combination of chemical and mechanical factors results in either irritation of the floor and sides of the ulcers, and with increased production of scar

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tissue, whence the callous ulcer—or further digestion of this inflamed, and, therefore, less resistant tissue, whence the progressive chronic ulcer and all its sequelae.

Such an interpretation accounts for the healing of ulcers of the fundus. Two factors are concerned in this. The first is the difference in peristaltic activity between the fundus and the antrum, the former acting simply as a reservoir in which the food is not thoroughly mixed with gastric secretion and from which the contents are slowly pressed out into the active right half of the stomach.[2] Secondly, it has been repeatedly demonstrated that food in the fundus is only faintly acid, or even alkaline, while always strongly acid in the antrum of the normal stomach, that is, the gastric juice is practically inert in the fundus, so that from this fact also fundal ulcers tend to heal.

To apply these facts to ulcer in man: As to the first factor, we need only say that normal mucosa is, of course, not digested, and that the tissue must first be injured in some way—whether by bacterial emboli, by toxic products, as in burns or uremia, by trauma, or by any of the processes which cause gastritis. As to the second factor, we have used the term "an active gastric juice" advisedly because there is no reason why chronic ulcers should not be produced by normally acid juice acting on diseased tissue, if the other factors are present, and such is the fact in some of the cases of our series, as in Cases 36 and 43 of Table 1, and, of course, in over 40 per cent. of clinical cases. In describing the third factor, we spoke of prolonged or vigorous contact, or both, because we can readily see why an abnormally vigorous peristalsis, even acting for a normal period, should produce the same results as a less active for a longer period. Of course, in our work the peristalsis is both exceedingly vigorous and prolonged, as we demonstrated fluoroscopically in a

2. Cannon: The Mechanical Factors of Digestion, p. 69.

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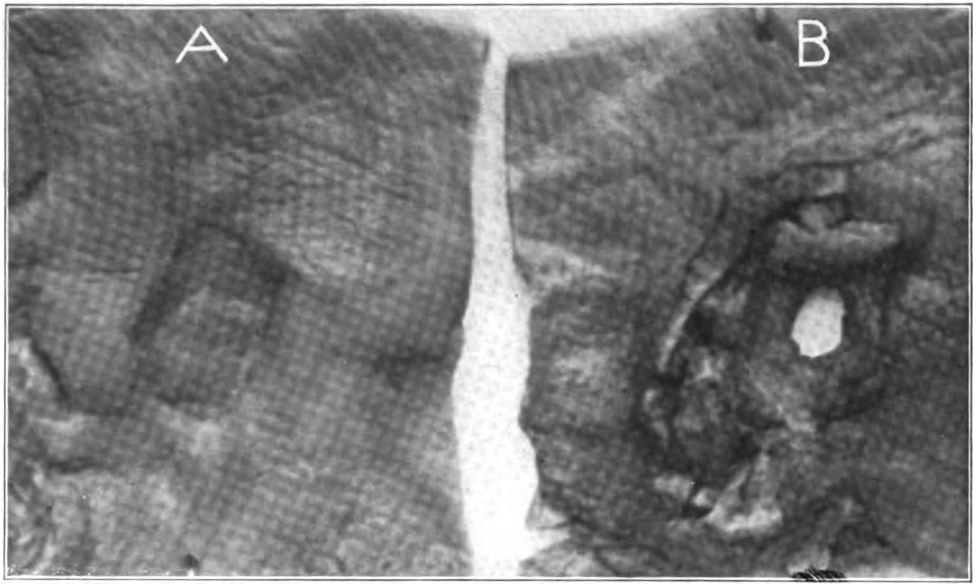

Fig. 1.—Acute ulcers, eight days after second operation.

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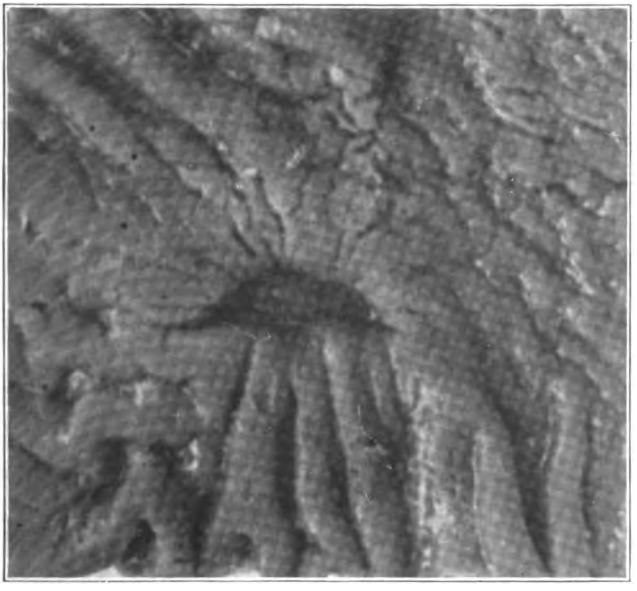

Fig. 2.—Chronic ulcer (delayed healing), three weeks after second operation.

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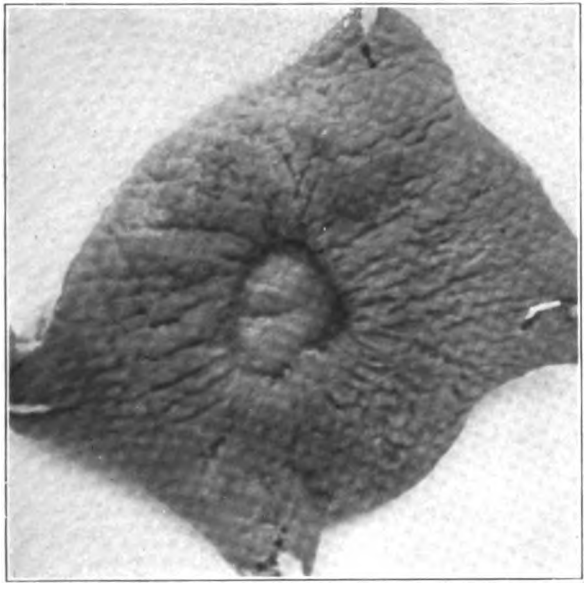

Fig. 3.—Chronic ulcer, five weeks after second operation.

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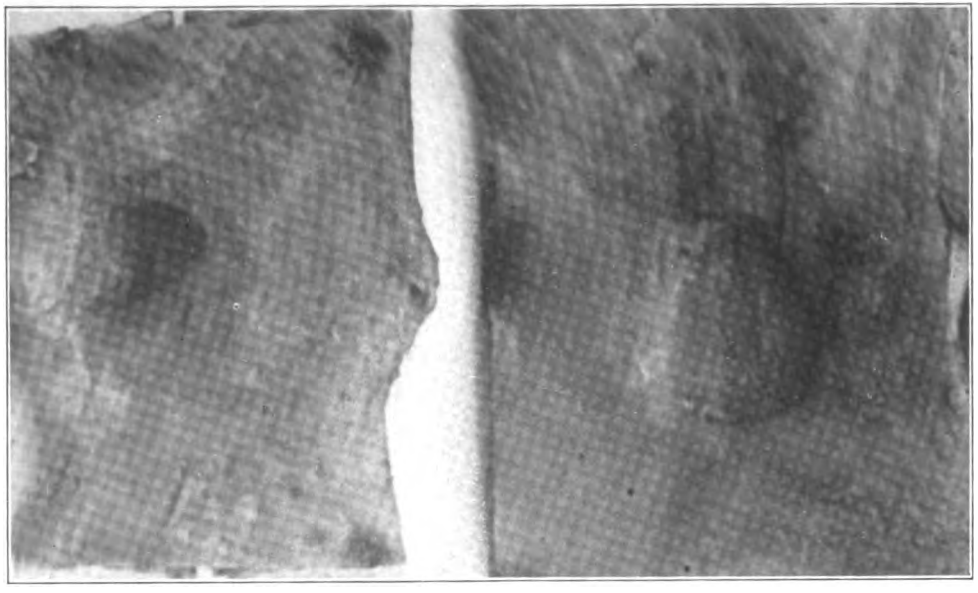

Fig. 4.—Chronic ulcers, five and one-half weeks after second operation; progressive.

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Fig. 5.—Chronic ulcer; markedly indurated base eight weeks after second operation.

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number of our cases. Clinically, such prolonged contact is certainly present in a majority of the cases of ulcer of the stomach. For instance, Smithies[3] reports on 404 cases of ulcer of the stomach verified at operation, in 65.3 per cent. of which there was definite abnormal food retention. The abnormally vigorous contact as a furtherance to autodigestion is supplied, first, by the hyperperistalsis due to pylorospasm, which is seen fluoroscopically not infrequently in injuries to the mucosa anywhere in the gastro-intestinal tract, and, secondly, by the hour-glass contraction (also spasmodic) found by Barclay,[4] also fluoroscopically, in ulcer of the body of the stomach in 90 per cent. of the cases. This spasmodic narrowing would, of course, increase the friction of the food particles. That such friction plays some part in human ulcer is shown by Stromeyer's[5] studies on the typical funnel shape of ulcer, with the proximal end deeper and the mucosa undermined, the distal end being shallowed and terraced, a fact which he explains by the progression of the stomach contents toward the pylorus, and its glacier-like, eroding (that is, digestive) action. This we found in a number of our ulcers, as in Figure 5 *A*, an ulcer of five and one-half weeks' duration, situated 4 cm. from the pylorus, in which one end was quite deep, with undermined mucosa, toward the pylorus. The shallowed and terraced end was nearer the cardia, the opposite of Stromeyer's statement, to be sure, but natural when we remember that the progression of food in the antrum is not in an uninterrupted straight line toward the pylorus, but oscillatory first in one direction, then in the other, and that, therefore, the undermining by digestion may occur at either end.

As a working hypothesis consistent with these experimental data, we may suppose either that acute ulcera-

3. Smithies: Gastric Ulcer without Food Retention, Am. Jour. Med. Sc., March, 1913, p. 340.
4. Barclay: The Stomach and Esophagus: A Radiographic Study.
5. Stromeyer: Die Pathogenese des Ulcus Ventriculi, Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), liv, 1912, No. 1.

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tions arise in the stomach from some outside cause and result reflexly in a pylorospasm, which again causes impaired motility, hypersecretion and hyperperistalsis, thus inducing chronicity; or that various conditions in the gastro-intestinal tract, as septic conditions in the mouth, mucous colitis, appendicitis, cholecystitis, etc., reflexly cause spastic contractions of various parts of the stomach, and narrowing of the lumen. If an abrasion occurs at the narrowed portion, for instance, from a coarse particle of food, the same process of digestion causes chronicity and the ulcer then perpetuates the spasm; or, more broadly, those persons whom we formerly termed neurasthenics, but who are now classed as vagotonics, may have pylorospasms from reflex causes in any part of the nervous system, and so the chain of events be set in motion (Barclay,[4] von Bergmann[6]).

SUMMARY

1. In the dog, chronic ulcers may arise from acute ulcerations, if certain factors are present, namely, prolonged and probably violent action of active gastric juice, that is, hypersecretion and hyperperistalsis, and that such ulcers may remain unhealed if the acidity later drops to normal. Other factors, such as anemia, absence of anti-pepsin, vascular obstruction, etc., are unnecessary presuppositions.

2. Stromeyer's theory of the digestive action of the gastric juice and the progress of food particles as determining the typical shape of ulcers is probably correct.

3. The majority of chronic ulcers are single and situated near the pylorus, because ulcers elsewhere tend to heal, the factors causing chronicity being usually present only near the pylorus.

29 East Madison Street—104 South Michigan Avenue.

6. Von Bergmann: Das spasmogene Ulcus Pepticum, München. med. Wchnschr., 1913, No. 4.

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ABSTRACT OF DISCUSSION

Prof. A. J. Carlson, Chicago: I observed a couple of years ago that about 75 per cent. of the dogs in which experimental parathyroid tetany had been produced showed certain types of ulcers in the duodenum and the pyloric mucosa. I thought at that time it indicated some relation between the parathyroids and these ulcers, as has been suggested by one of the previous speakers with reference to the internal secretions in general, but in extending the observations to cats I was quickly disabused of that premature conclusion in that I have never observed that phenomenon in cats in parathyroid tetany. I am inclined to believe that, of all the experimental animals, the dog is the one most prone to develop ulcers in the duodenum and in the pylorus without direct experimental interference that will tend to produce such ulcers. I do not mean by this to throw any doubt on the actual facts so beautifully shown by Dr. Rosenow with his streptococci injection or those reported by Dr. Friedman here to-day, but conclusions as regards the connection between the internal secretions and duodenal ulcers cannot be based on experiments on dogs alone.

Dr. E. C. Rosenow, Chicago: How long were these ulcers observed and were any cultures made from the depths of the ulcers which were produced experimentally? The results show clearly that obstruction of the pylorus in dogs delays the healing of ulcers produced by injections of silver nitrate and emphasize the rôle which obstruction undoubtedly plays in delaying healing of some ulcers in man; but why might not this be due to secondary infection of the walls of the ulcer made possible by rather than due directly to the interference with the function of the stomach? This it seems to me is a possibility which must be considered in view of the fact that the ulcers which I have produced by intravenous injections of streptococci from various sources, but of a particular grade of virulence and commonly with streptococci isolated from the depths of typical chronic indurated ulcers of the stomach and duodenum in man, tend to persist for a long time, occur most often in the pyloric end or duodenum, become indurated, may perforate or produce fatal hemorrhage and yield streptococci often in pure form as long as fourteen weeks after a single injection. Moreover, persistence of ulcers in man is not so uncommon when little or no disturbance of motor or secretory functions of the stomach can be made out.

Dr. J. C. Friedman, Chicago: First, as to Dr. Rosenow's question, the oldest chronic ulcer that I have is of ten weeks' duration. Of course, it is difficult to keep the dogs alive for a much longer period in a closed space. They get mangy and die spontaneously if we don't kill them. Secondly, we didn't

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make any cultures because we did not think that this was an etiologic factor in our ulcers. The reason why in cats one cannot produce ulcers as easily as in dogs may be connected with the different varieties of food that they eat. Surely, in the human, hyperacidity is present in the majority of cases of ulcer. Statistics show that hyperacidity is found in from 50 to 60 per cent. of the cases, and when we work experimentally we must take this functional change into consideration, and it may be that in Dr. Carlson's work these cats did not have any hyperacidity, while the dogs in the same experiments did. Therefore, the ulcers healed in the one and did not heal in the other. Now it is a known fact that one can produce acute ulcers in dogs and it is equally certain that in two or three weeks these ulcers heal and that therefore there must be some different etiologic factor in the ulcers which do not heal. As far as cutting out the suprarenal is concerned, not only do we destroy the internal secretions of that organ, but we also cause a vast change in the sympathetic system. Now it may be that the changes in the sympathetic system caused the ulcers without aid from the internal secretions.

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STUDIES IN INTESTINAL OBSTRUCTION

WITH A REPORT OF FEEDING HETEROLOGOUS JEJUNAL AND ILEAC CELLS TO A HUMAN BEING *

JOHN WILLIAM DRAPER, M.D.
NEW YORK

To Roger we are indebted for the present conception of the cause of death in intestinal obstruction. He was undoubtedly the first to conceive of it as a true auto-intoxication.

In a paper read before the Johns Hopkins Medical Society seven years ago and based on a study of the effects of 400 duodenal obstructions produced in animals at the Surgical Research Laboratory at Columbia, I reported corroborative evidence, from a surgical aspect, of Roger's view. William Welch on opening the discussion of my paper said that, if corroborated, the facts presented would show that the duodenum had a function hitherto unknown and one evidently of the utmost importance in medicine. It is gratifying that this corroboration has just recently come from Dr. Welch's own laboratory at the hands of Whipple and his associates. By a series of skilfully devised experiments, these investigators have succeeded in casting much more light on this interesting problem than either Roger or I had been able to do, and the sum of our investigations taken in the aggregate seems now to offer incontrovertible proof that the cause of death is not bacterial in origin but truly autotoxic from the cells of the epithelium of the intestine itself.

* From the Laboratory of Experimental Surgery, New York University.

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Perhaps no branch of medical research better illustrates than this one the advantages derived from having men with one object in view working at different centers of education toward the solution of a common problem. The different points of view ultimately arrange themselves on convergent lines so that when the answer is finally won it is evidently not the product of one man's aims or ambitions, but a homogeneous unit, resulting from the constructive work of several minds. For no single individual can expect to do much more with a modern problem than to advance it a single step. This problem also serves to demonstrate the rapid breaking down of the traditional barriers between medicine and surgery, forecasting the ultimate fusion of these old and arbitrary subdivisions into a new unit, the members of which will have a working knowledge of physiologic chemistry and its allied sciences in addition to requisite technical skill. Furthermore, it demonstrates that modern surgery, like physiology, does not consist in the manufacture and use of instruments of mechanical precision but in philosophical interpretation of natural phenomena.

The various old and groundless theories as to the cause of death in intestinal obstruction have one by one been disposed of, with the possible exception of the exact origin of the toxemia. Is it bacterial, or purely autotoxic from the cells of the intestinal epithelium itself? Were it not for the views of Fred Murphy, which result from his studies at the Washington University and which bear evidence favorable to the bacterial theory, one would be ready to accept the result of the painstaking and thorough researches at the Cornell laboratories of Hartwell and Hoguet who seem definitely to have excluded the anaerobes, and of Whipple and his associates at the Hopkins laboratories, who are disposed to rule out all bacteria as causative agents in the development of the toxemia.

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From the prosaic and common sense point of view of applied surgery, it is hard to understand why obstruction in the duodenal region should be many times as dangerous as obstruction in the colon. It has long been known that duodenal contents may be allowed to escape into the peritoneal cavity without danger, but that peritonitis is to be expected if any colonic contents escape.

No careful observer could watch the death of a series of duodenally obstructed dogs without noticing the close similarity between the ante-mortem symptoms of this condition and those seen after parathyroidectomy or long remain in doubt that the function of the liver was greatly impaired in each.

The accompanying tables are based on the study of the urine of duodenally obstructed dogs, both before and after a large dose of camphor had been given hypodermatically. The recovery of the paired glycuronic camphor has been made by the method devised by Tollens. I am indebted to Frederick W. Schlutz of the University of Minnesota for the privilege of presenting these results, which are a chronicle of his chemical studies made on dogs obstructed by me. In a previous communication we have jointly presented two similar studies, both of which appear to show more conclusively than the present ones that liver function was impaired. Negative findings, reported of carefully wrought work, are, however, just as valuable as positive findings, particularly in the case of a method which is as yet open to some question as to accuracy, and which is certainly open to criticism as regards the time taken in working it out. Dr. Schlutz is carrying on further studies of the accuracy of Tollens' method. As indicated in Table 1, the discrepancy between this report and our previous findings, in which the liver function, as indicated in terms of camphor-glycuronic was considerably lowered, may be due either to inaccuracies in the

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method itself or our application of it, or to the fact that we were unfortunate in having one of our dogs live but a very short time while the other one lived an unusually long time. Dr. Schlutz will make a further report which will clarify this interesting question.

TABLE 1.—INTESTINAL OBSTRUCTION, DOG 201: LIVER FUNCTION IN TERMS OF GLYCURONIC CAMPHOR (TOLLENS), SCHULTZ-DRAPER SERIES

Date of Experiment	Length of Period, Hrs.	Vol. of Urine, c.c.	Normal Amt. of Glycuronic Acid	Amt. actually Passed as Combined Camphor Glyc. Acid	Increase Over Normal in Glycur.	Remarks
1-27-28	24	130	0.1794	.....	.....	2 gm. of camphor.
1-28-29	24	122	0.1092	.....	.....	A Theoret. total of 452 gm. Comb. camph. glyc. acid. 37.02 per cent. combined before, 5.64 per cent. obstruction.
	Camphor 2 gm. hypod. 3 p. m. ½ 9-1912					
1-29-30	24	147	0.1470	0.4428	0.2958	1 c.c. of normal undiluted urine = 0.0010 gm. glyc. acid.
1-30-31	24	215	0.2150	1.4094	1.1944	1 c.c. of normal diluted urine = 0.00028 gm. glyc. acid.
1-30-11-1	25	227	0.2270	0.4104	0.1834	
	Intestinal obstruction, 3 p. m. 7-1912; 24 hrs. later 2 gm. camphor hypodermically.					
11-1-2	34	900	0.9200	1.1772	0.2572	

Discrepancy with results previously reported may be due to short post-operative life or to inaccuracy of Tollens' method.

When Hartwell and Hoguet advanced their hypothesis that water privation was the chief lethal factor after intestinal obstruction, at the suggestion and with the help of George Wallace we conducted some experiments to determine the exact water percentages in the tissue of dogs under various conditions. It was thought that salivation by pilocarpin,

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fasting, and duodenal obstruction might cause approximately the same water-loss to the tissues. Table 3 shows the results from six tissues, namely,

TABLE 2.—INTESTINAL OBSTRUCTION, DOG 202: LIVER FUNCTION IN TERMS OF GLYCURONIC CAMPHOR (TOLLENS), SCHLUTZ-DRAPER SERIES

Date of Experiment	Length of Period, Hrs.	Vol. of Urine, c.c.	Normal Amt. of Glycuronic Acid	Amt. actually Passed as Combined Camphor Glyc. Acid	Increase Over Normal in Glycur.	Remarks
1-27-28	24	116	0.1458	.....	.....	1.7 gm. camphor = a theoret. total of 3.842 gm.=comb. camph. and glyc. acid. 0.2 gm. camphor = 452 gm. comb. camphor and glyc. acid.
1-28-29	24	150	0.1548	.....	.....	
	1.7 gm. camphor in oil subcut.					
1-29-30	24	83	0.0913	1.2294	1.1381	Before obstruc. 44.56 per cent. of camphor comb. with glyc. acid.
1-30-31	24	100	0.1100	0.3582	0.2482	After obstruc. 47.64 per cent. were comb., but practically all in the first 24 hrs. after that markedly dim. function.
1-31-2-1	24	130	0.1430	0.2880	0.1450	1 c.c. of normal undiluted urine = 0.0011 gm. glyc. acid.
	Intestinal obstruction 4:30 p. m. 2/1/12.					
2-1-2	24	55	0.0615	0.2424	0.1809	
	Camphor 2 gm. subcut. 2/2/12, 2 p. m.					
2-2-3	24	366	0.4026	2.3100	1.9074	
2-3-4	24	109	0.1199	0.2310	0.1111	
2-4-5	24	246	0.2706	0.2906	0.0200	
2-5-6	24	134	0.1474	0.2208	0.0724	
2-6-7	24	85	0.0935	0.1140	0.0205	
2-7-8	24	60	0.0660	0.0888	0.0228	

Postoperative life unusually long may indicate high liver resistance or inaccuracy in Tollens' method.

liver, lung, heart, kidney, spleen and muscle. It is seen that within a few tenths of 1 per cent. the water-loss after pilocarpin, after fasting, and after death from duodenal obstruction was the same, namely,

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TABLE 3.—WATER PERCENTAGES OF SALIVATED AND DUODENALLY OBSTRUCTED DOGS ACCORDING TO TISSUE; SALIVATED SERIES, EUTHANASIA FOURTH DAY; FASTING, EUTHANASIA SEVENTH DAY; OBSTRUCTION SERIES, AUTOTOXIC DEATH ON FOURTH DAY (AVERAGE)

Tissue	Normal					Salivated (Pilocarpin)			Fasting 7 Days	Duodenally Obstructed					
	Dog	211	193	236	Aver.	205		Aver.	204	207	206				Aver.
Liver..	73.6	72.0	70.0	79.2	73.7	74.8	60.0	67.4	62.6	59.0	60.6	69.0	68.0	71.0	65.5
Lung..	....	80.4	68.2	84.3	77.6	71.2	79	75.1	76.8	72.8	66.8	....	....	....	69.8
Heart..	77.0	77.0	84.0	75.0	78.2	73.0	63.0	68.0	74.8	74.4	62.0	55.0	75.0	77.0	68.7
Kidney.	....	78.7	80.0	74.5	77.4	75.2	....	75.2	70.9	67.2	59.3	....	74	78	69.6
Spleen.	....	76.3	77.5	74.1	75.9	....	51.0	51.0	....	64.5	....	....	74.0	79.0	72.5
Muscle.	75.0	74.0	75.1	72.7	74.2	44.2	74.0	59.1	45.6	42.8	40.0	65.0	69.2	....	54.2
Average	....	....	....	....	76.1	....	....	66.0	66.1	....	....	....	....	....	66.7

Salivation, fasting and duodenal obstruction each cause 10 per cent. water loss, showing this factor to be negligible in death by obstruction.

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10 per cent. In the case of the salivated dogs and the fasting dogs, euthanasia was practiced on the fourth and seventh day, respectively, long before there were any signs of disability. On the other hand, the duodenally obstructed dogs were allowed to die of their autotoxemia, so Table 3 gives the maximum water-loss for obstruction but not for salivation or for fasting.

These studies appear to offer additional proof that in intestinal-obstruction toxemia, as in other toxemias, whether bacterial or truly autotoxic in origin, the solvent power of water on the toxins and its help in eliminating them from the body serve in many cases to prolong life, and, under certain conditions, to save it. Indeed, Wallace has shown that an animal may be given double the lethal dose of diphtheria toxin and yet live, if treated by abundant hypodermoclysis. But while valuable from a therapeutic point of view they cast no light whatever on the origin or the nature of the toxins. Furthermore, it has been the clinical experience of all surgeons that even an abundance of water will not serve to save the life of a human being in whom there had existed for any length of time complete duodenal obstruction.

We have again subjected to careful microscopic study the liver, heart and kidneys of a large number of obstructed animals and find that except for capillary dilatation they appear normal. In this connection as well as in the conduction of the feeding experiments, I acknowledge the efficient help of Dr. Eisberg. These microscopic findings are simply corroborative of previous reports made by myself and others. The beginning and end of the alimentary canal, however, on its epithelial surface, shows this capillary dilatation so markedly as to attract the eye on gross examination. I have shown a colored photograph of a stomach removed from a duodenally obstructed dog at the Mayo Clinic. I have shown and called attention to this before, without knowing its importance

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or significance; and because of its bearing on certain data which I shall present later, I am glad to say that the observation is corroborated by Whipple. In our necropsy experience the small intestine has shown little if any, gross change, but the colon is almost invariably affected and in a very characteristic manner. I understand from Wallace that the same condition obtained after a lethal dose of diphtheria toxin had been given.

Thus it appears increasingly probable that the toxins of intestinal obstruction are eliminated both from the stomach and from the colon. When we recall the method of elimination by the stomach of morphin, its reabsorption by that organ and the value of frequent stomach-washings in the treatment of morphin poisoning, the post-mortem picture of intestinal obstruction is not so surprising, and the value of lavage and epithelial cell feeding in complete and incomplete obstruction as well as in acute dilatation of the stomach is more easily understood. It strongly suggests that most cases of postoperative dilatation are due to an adynamic duodenum, just as hydronephrotic dilatation of the kidney is often caused by physiologic ureteric obstruction. An organ dilates first at the point of obstruction to its outlet, be the obstruction mechanical or physiologic.

The capillary evidence of stomach elimination affords a working hypothesis on which we may explain the interesting results of feeding to duodenally obstructed animals the epithelial cells from the ileum and jejunum of other animals. Table 4 shows the result, as regards the length of life, to be quite definite, the fed dogs living nearly twice as long as the controls. This series agrees with a similar series reported by me from the Mayo Clinic. It is not to be forgotten that scrapings from the frog's duodenum will stimulate pancreatic secretion in the higher vertebrates. Intercellular reactions must be among the most ancient and most fixed of our functions, and

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the feeding of intestinal cells may prolong life by reestablishing certain vital intercellular reactions which the obstruction had interrupted. Although knowing empirically that if jejunal and ileac epithelium were placed in the stomach of a duodenally obstructed dog, that dog would outlive one either not fed on cells at all or fed on cells of organs other than the small intestine. I could not previously understand this but it now seems probable that since the

TABLE 4.—FEEDING HOMOLOGOUS CELLS TO DUODENALLY OBSTRUCTED DOGS

Fed Jejunal and Ileac Epithelium			Fed Emulsified Liver, Spleen, Kidney, Pancreas and Muscle Tissue			Not Fed (Controls)		
Dog No.	Hours Lived After Obst.	Average Pulse	Dog No.	Hours Lived After Obst.	Average Pulse	Dog No.	Hours Lived After Obst.	Average Pulse
200	197	157	49	72	156	185	83	149
202	114	147	53	115	140	186	239	109
204	171	161	56	75	149	188	66	133
33	166	200	57	71	179	189	50	167
34	94	136	58	147	155	191	126	174
38	284	182	63	74	171	192	53	134
48	72	144	64	52	163	193	108	170
44	191	140	65	47	140	196	68	154
45	101	150	62	144	181	197	72	141
46	258	143	59	247	140	198	20	172
	1648 164.8*	1560 156†		1044 104.4‡	1574 157.4¶		885 88.5§	1563 156.3‖

* Average hours of life when fed epithelium.
† Average pulse when fed intestinal epithelium.
‡ Average hours of life when fed emulsion of organs.
¶ Average pulse when fed emulsion of organs.
§ Average hours of life of control.
‖ Average pulse of control (at femoral).

toxins are undoubtedly thrown out into the stomach they are directly rendered harmless by the heterologous cells from the small bowel derived from a healthy animal and are thus prevented from reentering the body. This explains the immediate benefits derived in the treatment of acute dilatation of the stomach and since this condition is undoubtedly due to physiological duodenal obstruction, it seems probable that

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the feedings of these epithelial cells may prove to be just as useful in the treatment of dilatation as in the postoperative treatment of obstruction.

The study of Table 4, constructed from the last series, shows that it differs from the former series in that the pulse-rate of the different groups has been unaltered. A reasonable explanation of this is that the pulse was taken in the recent series at the femoral, whereas in the series reported from Rochester it was in each case taken at the apex. The femoral count is certain to be much lower when the heart is running high because many of the beats are not transmitted to the finger. While this series for the foregoing reason shows little change in the pulse-rate, it does show a much greater increase in the length of life, and this certainly is the best index of the effect of any treatment.

A glance at Table 4 shows that of the dogs fed with epithelium only two died before the hundredth hour, whereas of the controls only three lived beyond the hundredth hour. It is interesting to note that one of these lived 239 hours, showing that occasionally one finds an animal naturally resistant to duodenal obstruction. In the average this is balanced in the fed series by one which lived 284 hours, so that the general average remains unchanged and it is quite safe to say that the increase of life, which in this series is nearly doubled, is due entirely to the detoxicating effect of the epithelium.

In order to show that fed jejunal and ileac epithelium exercise some special detoxicating power not yet understood but definitely recognizable, we fed a control series of dogs on the emulsified cells of liver, spleen, pancreas and muscle-tissue. These animals lived a few hours longer than the not-fed controls, but it is evident that these cells had either no detoxicating action, or a very feeble one, compared with the intestinal epithelium. If we prove to have at hand some less cumbersome method than Tollens' for

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determining liver function, it would be interesting to use it as an index of the effect of feeding small intestine epithelium.

I have utilized jejunal and ileac epithelium clinically in two instances. First in a valuable female dog which had been imported from England about six months before, and had had "chronic stomach trouble" ever since her arrival. She, however, whelped a large litter of pups about six weeks before I saw her, without difficulty. She was a large poodle, weighing about 40 pounds. She had had complete intestinal obstruction for five days and she presented all the clinical symptoms which I have described, notable tachycardia, extreme nervousness, and great weakness in the hind extremities. On opening her, with the consent and on the advice of Dr. Blair, I removed from the oral portion of the small intestine and at a point not exactly determined, the pebble which is shown in the illustration. Her condition was very grave, the pulse running from 180 up and showing great irregularity at the apex. She was immediately put on small intestine epithelium derived from two dogs of a different breed. No one is justified in drawing any deductions from one case, but from a long experience with the symptoms of duodenally obstructed dogs, I should not under ordinary conditions have expected her to recover. The symptoms, however, gradually subsided and she lived.

The second instance in which I have used the epithelium therapeutically was in the case of a man referred to me by my colleague Jerome Lynch. The patient had had definite symptoms of obstructions for ten days, and when the abdomen was opened, an annular cancer was found which closed the terminal ileum. Not knowing whether heterologous epithelium would have any detoxicating effect, but remembering that the duodenal epithelium of frogs is capable of stimulating pancreatic secretion in the higher vertebrates, and realizing that this patient was

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in a desperate condition, Dr. Lynch and I decided to feed him the emulsion from a dog. Hourly doses were given until the entire epithelium from the two animals had been taken. The patient objected much more to some magnesia which we ordered, than to the dose of epithelium, which he described as "not half bad." The pulse improved and the patient lived. These cases prove nothing; they simply show that heterologous cells may be given without discomfort or harm.

Different operative procedures were used in each. In the first, the continuity of the bowel was reestablished and most of the pent-up products of obstruction were allowed to flow to the aboral portion of the intestine. In the second, owing to the patient's condition, it was found necessary to do an ileostomy, and all the obstruction products were drained to the surface. As I have already stated, it is by no means proved that because such products are toxic to other individuals, they are necessarily toxic to their host.

CONCLUSIONS

The power of the liver to pair camphor and glycuronic acid is probably seriously impaired after duodenal obstruction. This can be studied by the method of Tollens, which, however, is cumbersome and may be faulty. Such decreased power of camphor-pairing is presumably an evidence of impaired liver function. This, however, is not reflected in the histologic appearance either grossly or microscopically.

The decrease in the water-content of the tissues in duodenal obstruction is about the same as obtains after salivation by pilocarpin for four days or after fasting for seven. As this decrease produced no visible change in either case before euthanasia, it is reasonable to believe that it produces none in intestinal obstruction. The loss is 10 per cent.

The toxemia in duodenal obstruction undoubtedly arises from an interference with cellular reactions of

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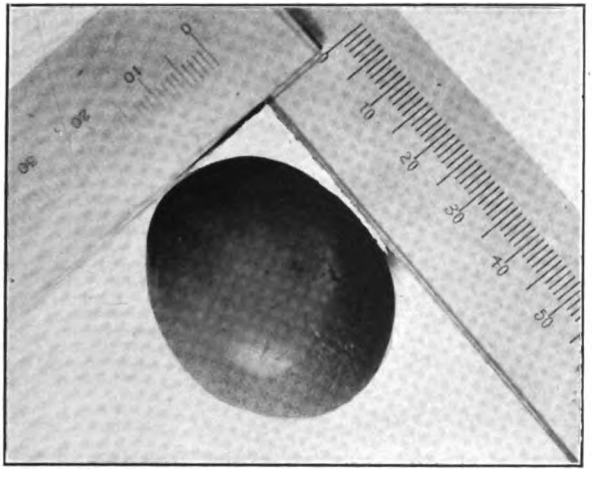

Acute jejunal obstruction in dog. Stone swallowed six months before operation, causing chronic stomach symptoms, but no obstruction until it slipped into the bowel. Note partial digestion of soft area by gastric juice. Recovery followed removal of pebble and feeding of dog with jejunal and ileac epithelium.

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the intestinal epithelium. The toxins undoubtedly are at least in part eliminated from the stomach and colon. If small-intestine epithelial cells of healthy animals are placed in the stomach of duodenally obstructed animals, such animals have lived nearly twice as long as not-fed control animals. This evidence is strongly opposed to the bacterial theory of origin of the toxins.

In addition to the placing of jejunal and ileac epithelium in the stomach of postoperative obstruction cases, an emulsion of them should also probably be used in colonic irrigations for the same indication and purpose.

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ABSTRACT OF DISCUSSION

Dr. Joseph Colt Bloodgood, Baltimore: Dr. Draper's paper seven years ago made me think clinically about the duodenum in relation to some things we had observed in intestinal obstruction. In the beginning of surgery, we were dominated by anatomy, and then followed pathology. At present surgery does not look for much help to anatomy, except in certain regions, or even to pathology. We have about reached the limit of the microscope, but we do look for help to experimental physiology in the solving of problems which will be met on the operating-table. Perhaps, later, we will look to experimental chemistry. Surgeons, as soon as they conquered technic in surgery of the stomach, found that their patients did not die of peritonitis. As soon as they used anesthesia, the majority of their patients didn't die of shock and later of anemia, but up to the present there is one complication which has always bothered. If they do a posterior gastro-enterostomy a number of their patients vomit the contents of the duodenum, because of some fault in the technic. Now its contents are washed out with the stomach-tube. These patients in the beginning died because we didn't know how to use the stomach-tube. There was no place for the duodenal contents to flow. They couldn't pass up into the stomach and couldn't come through the anastomosis because it was kinked, and these patients died within from three to five days. When I heard Dr. Draper's paper I couldn't understand some of the mortality after operations of that kind, either of my own or others. Patients going well on the operating-table, apparently absolutely comfortable for twenty-four or thirty-six hours, suddenly going to pieces, with no pumping, no distension or rapid pulsing that show in intestinal obstruction. That is a definite case of death from acute dilatation of the duodenum as a definite postoperative complication. If there is a patent pylorus

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we can relieve that with a stomach-pump when the pylorus is closed by section. Some surgeons are now advocating that posterior gastro-enterostomy is without an element of danger, and will not be followed by complications in two or three days. The operation has led to chronic dilatation of the duodenum, a terminal condition which is responsible for the ill-health of a group of patients. In the last three years I have recognized and operated on six. In the previous three years I didn't recognize three. The previous three died. The subsequent six have recovered, and the cause of the dilatation I have found has been a congenital defect in the mesentery to the ileum from the appendix and the giant cecum descending into the pelvis, and on account of the short mesentery of the ileum lying directly on the base of the mesentery section, which kinks the small intestine as it passes through to a junction with the duodenum, which produces a chronic dilatation with its symptoms. These patients have been relieved by removing the colon and taking away that heavy part and exposing the transverse colon so that it will do its work and be allowed to expand. This is a demonstration of what experimental physiology can do for practical surgery.

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ROENTGENOLOGIC OBSERVATIONS ON THE FUNCTION OF THE ILEO-COLIC VALVE

WITH SPECIAL REFERENCE TO THE CAUSATION OF ILEAC STASIS

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The subject of intestinal stasis, particularly ileac stasis, has recently aroused much interest and not a little controversy. Most of the discussants are agreed as to the significance of ileac stasis, but the mechanism of its causation, and, consequently, the choice of methods aimed at its relief, has been the subject of much theorizing and controversy. Enteroptosis, adhesion bands, various pericolonic membranes, intestinal kinks, cecum mobile, and typhlatony have been among the causes named; but the instances in which ileac stasis exists without any demonstrable bands or kinkings are so numerous it seems some other cause must be found to explain the stagnation.

It is, therefore, important to determine, if possible, the correct basis on which to build the argument relating to the causation of ileac stasis. If the case be the kinking of the terminal ileum or some similar lesion and it is conceded that, in comparatively rare cases, obstructing kinks and adhesions do occur, careful consideration must be given the recommendations for serious surgery which seem to be indicated. On the other hand, if the ileac stasis be dependent on some other and simpler cause as, for instance, ileocecal

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sphincter spasm or incompetency of the ileocecal valve, there will be little indication for hazardous surgery.

For several years (since 1910) I[1] have been urging ileocolic valve incompetency as an explanation of a large share of these cases of ileac stasis. In September, 1912, I reported a series of 200 gastro-intestinal cases,[2] most of the patients being constipated, in which 33, or 1 in 6, showed ileocecal valve incompetency when tested by the bismuth enema. In August, 1913, I reported a much larger series of 1,500 persons,[3] tested by means of the bismuth enema, in which nearly 250, or again 1 in 6, showed ileocolic valve incompetency. It should be emphasized that this large series of patients were all suffering from gastro-intestinal symptoms and most of them were seeking relief from constipation. I have now found more than 500 cases of ileocolic valve incompetency, the frequency of occurrence remaining about 1 in 6 of the cases examined.

The anatomists describe the ileocolic valve as formed by partial inversion or telescoping of the ileum into the cecum. The end of the small intestine is invaginated through the wall of the large bowel, carrying with it certain layers of that wall which project into the cecum as two folds, lying, respectively, above and below its orifice and constituting the two segments of the ileocecal valve. The peritoneum and the longitudinal muscular fibers of the bowel do not take part in this infolding, but are stretched tightly across the crease produced on the exterior by the inversion, thus serving to preserve the fold and the formation of the valve. According to Cunningham, whose description has just been given, the efficiency of the ileocolic valve is largely due to the oblique manner in which the ileum enters or invaginates the cecum, and he states that, in the great majority of

1. Case, James T.: Proc. Am. Roentgen-Ray Soc., 1911.
2. Case, James T.: Tr. Am. Roentgen-Ray Soc., September, 1912.
3. Case, James T.: Proc. XVII. Internat. Cong. of Med., Section on Radiology, London, August, 1913; also in Med. Rec., March 7, 1914.

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instances, when in position within the body, the ileum is perfectly protected from a return of the cecal contents. In fact, anatomists are almost unanimous in thus considering the normal ileocolic junction a barrier against a reflux of cecal contents.

According to Arthur Keith[4] there are muscular fibers situated directly at the ileocolic orifice which are evidently designed to secure that orifice from reflux from the cecum. In addition to the sphincter action of the circular muscles included in the valve itself, the last few centimeters of the terminal ileum present a distinct thickening of the circular muscular coat which seems to be endowed with a special tonic function; it serves as a sphincter for the terminal part of the ileum. This sphincter relaxes in front of the peristaltic wave, allowing the passage of food into the colon, but, on the other hand, it contracts, as a rule, against any regurgitation which might be caused by contractions in the colon. In marked contrast to the rest of the bowel in its relation to the central nervous system, the ileocolic sphincter is not affected by vagus stimulation. According to Elliott, stimulation of the splanchnic nerves causes complete relaxation of the terminal ileum and produces a strong contraction of the ileocolic sphincter. These statements are based on the current teachings relating to the anatomy and physiology of the ileocolic junction.

It thus appears that the ileocolic junction is possessed of a double function, namely: (1) a sphincter action which controls the passage of ingested food from the ileum into the colon and tends to protect the ileum from a reflux of cecal contents; (2) a valve action which is probably purely mechanical, guarding against reflux back into the ileum. Much of the force of the arguments relating to ileocolic valve incompetency as a factor in the causation of ileac stasis hinges on the determination of whether or not the ileocolic valve is normally competent.

4. Keith, Arthur: Proc. Roy. Soc. Med., April, 1913 (supplement).

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Most anatomists and physiologists have thus far agreed that the valve is normally competent. The work of Cannon and others on antiperistalsis adds importance to the conception of normal ileocolic valve competency. Our present-day conceptions of the functions of the ileum and of the colon point to the need of such a barrier. My Roentgen-ray studies on colonic peristalsis and antiperistalsis confirm, almost to the minutest detail, Cannon's deductions from Roentgen-ray observations on animals.

Bull,[5] in 1878, after a careful series of experiments, stated his belief that, in the living body, fluid could not be forced beyond the ileocolic valve, but when the bowel was overdistended by air the valve was rendered incompetent and the air entered the small intestine. Dawson,[6] in 1885, experimenting on the cadaver, concluded that the ileocecal valve, when normal, effectually guards the small intestine from gas and fluid from below. Ziemssen[7] recommended inflation of the rectum as a means of diagnosis and treatment by "special procedure," the patient being placed under anesthesia. He states that ordinarily the small intestine is closed to the return of material from the cecum by the ileocolic valve, but under deep chloroform anesthesia this resistance is decreased and fluid can be forced into the small intestine. Heschl,[8] after a number of experiments, agreed with Ziemssen as to the normal barrier function of the valve.

On the other hand, a few observers, such as Cantani,[9] firmly believed that the ileocolic valve was permeable to fluid injections and suggested that the small intestine be utilized in cases of rectal alimentation. In one instance in which he treated acute bowel obstruction by injection of oil, half an hour later a part of the oil was vomited. Ziemssen records a

5. Bull: Virchow's Jahresbericht, 1878, ii, 205.
6. Dawson: Lancet-Clinic, Feb. 21, 1885.
7. Ziemssen, quoted by N. Senn: Experimental Surgery, 1889.
8. Heschl: Zur Mechanik diastaltischen Darmperforationen, Wien. med. Wchnschr., 1881, No. 1.
9. Cantani: Virchow's Jahresbericht, 1879, ii, 180.

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similar case in which a fistulous connection between the colon and the stomach was suspected because the patient vomited oil shortly after an oil enema. Hemmeter, Church, and Gruetzner[10] have all conducted experimental work which supports the idea that, in certain cases at least, the ileocolic valve permits the reflux of material from the colon. Yet Boyd[10] makes the statement that the reflux of fluid through the ileocolic valve cannot, in every case, be expected, and that he has frequently failed to find insoluble substances, such as charcoal, in the gastric contents, in cases in which charcoal had been added to the nutrient enemas.

Illoway[11] reports four cases of intestinal obstruction treated by forcing water past the ileocecal valve, with recovery in three cases. In this connection it is proper to refer to the experiments of Senn[12] who, discussing the use of rectal injections in overcoming bowel obstruction due to invagination, concluded that, when the obstruction is located beyond the ileocolic valve, no reliance can be placed on this method. Senn based this statement on carefully conducted experiments on animals in which fluid was forced beyond the ileocecal valve. In two cases post-mortem examination revealed longitudinal lacerations of the muscular coat of the rectum. A third animal died immediately after the experiment from the effects of the injection. Further, but less crucial, experiments were carried on in man. Senn concluded that "the forcible injection of water beyond the ileocolic valve must therefore be recognized as a dangerous expedient and should never be resorted to."

Senn further states, however, that, in the healthy adult, the competency of the ileocolic valve is overcome by rectal inflation under a pressure of from 1½ to 2¼ pounds, and that this amount of pressure

10. Quoted in Sutherland's System of Diet and Dietetics, 1908, p. 287.
11. Illoway: Am. Jour. Med. Sc., xli, 168.
12. Senn: Experimental Surgery, 1889.

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is not sufficient to injure the bowel. Behrens agrees with Senn in this statement. The incompetency of the valve is caused in cases of overdistention by a lateral and longitudinal distention of the cecum which mechanically separates the margins of the valve.

In 1897, Max Hertz[13] of Vienna, while practicing abdominal massage on a patient for abdominal pains, observed peculiar symptoms, which after further study on other cases, led him to describe a symptom-complex of insufficiency of the ileocecal valve. In 1910, and on numerous occasions since, I have demonstrated, roentgenologically, the retrograde filling of the ileum after bismuth clysmas. Holzknecht and Singer,[14] in 1911, mentioned the relatively frequent occurrence of ileocecal valve incompetency in connection with chronic obstipation. In 1912, Groedel[15] reported insufficiency of the ileocecal valve and submitted roentgenologic evidence. In 1913, Groedel[16] continued his discussion of ileocecal valve incompetency and there appeared papers by Dietlen,[17] Kellogg,[18] Reider, Schwarz,[19] Katsch[20] and myself.[21]

From certain quarters, however, have come expressions of doubt as to the serious significance of ileocecal valve incompetency, and skepticism has been expressed concerning the normal competency of the valve. I therefore wish to tabulate at some length a series of findings, most of them roentgenologic, which seem to indicate that the ileocecal valve is normally competent, protecting the ileum from a reflux of cecal contents.

1. The ileocecal valve is almost universally present in vertebrate animals (Kellogg[18]). I have found

13. Hertz, Max: Wien. med. Wchnschr., 1897, Nos. 36 and 37.
14. Holzknecht and Singer: München. med. Wchnschr., 1911, No. 48.
15. Groedel: Verhandl. d. deutsch. Roentgen Gesellsch., 1912, viii, 56.
16. Groedel: Fortsch. a. d. Geb. d. Roentgenstr., 1913, xx, No. 2.
17. Dietlen: Fortschr. a. d. Geb. d. Roentgenstr., 1913, xxi, 23.
18. Kellogg: Med. Rec., June 21, 1913.
19. Schwarz: Die klinische Roentgendiagnostik des Dickdarmes, Berlin, 1914.
20. Katsch: Fortsch. a. d. Geb. d. Roentgenstr., 1913, xxi, 177.
21. Case, James T.: Proc. Am. Roentgen-Ray Soc., 1914; New York Academy of Medicine, Jan. 15, 1914.

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the ileocolic valve of the dog and of the pig competent, withstanding enormous distention of the colon by gas. The following surgical operation on a dog is submitted as further evidence of the competency of the ileocolic valve, at least, in the dog. The pelvic colon and the ileum were so divided and reinserted that the course of the food was from the ileum into the descending colon, then through the transverse, the ascending, and through the ileocolic valve and the last 10 inches of the ileum into the pelvic colon and rectum. Dr. D. B. Phemister, who performed the operation, reported that the dog died of intestinal obstruction thirty-six hours later, intestinal contents having accumulated on the distal side of the ileocolic valve, which remained thoroughly competent.

2. A young man was given a silk string 9 meters long, one end of which he swallowed. At the end of twelve hours, the string had transversed the entire gastro-intestinal tract. By means of the bismuth enema, I tested the valve competency on two separate occasions before introducing the thread, and again after the thread was in place, and found that the valve was thoroughly competent. Then, for the purpose of an experiment on electrical stimulation of the bowel, the thread being used as a guide, a metal electrode was introduced as far as the upper end of the descending colon, incidentally considerable traction being made on the thread in an unsuccessful effort to pass the sound around the splenic flexure. Roentgenograms made a few moments later showed the terminal ileum filled for a considerable distance. This was attributed to traction on the thread, rendering it taut in the region of the valve, producing temporary incompetency. After the experiment was concluded and the thread withdrawn, another bismuth enema was injected, and the valve was again found to be competent.

3. In a certain number of cases, following the injection of a bismuth enema, the head of the bismuth

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column will pass the ileocecal valve and fill the terminal ileum for a varying distance. As already mentioned, I have found ileocolic valve incompetency in about one-sixth of my bismuth cases. This statement is based on the roentgenoscopic examination of more than 3,000 persons (June, 1914), most of them constipated, and all suffering from gastro-intestinal disturbances. Dietlen,[17] in 100 cases, found 22 in which the bismuth clysma showed ileocecal valve incompetency, 1 to 5. Holzknecht and Singer[14] found 3 cases of valve incompetency out of 15 cases examined, or 1 in 5. On the other hand, Dietlen observed insufficiency of the valve in only one of the normal cases which he examined during a period of several years. This agrees with my experience in examination of a number of presumably normal persons.

The method of testing the competency of the valve is the following: The patient should lie supine on the horizontal fluoroscopic table. It is not necessary to introduce the rectal point, or colon tube, more than 1 or 2 inches. The container should be placed not higher than two feet above the patient and the barium or bismuth enema allowed to flow by gravity, the course of the bismuth column being watched fluoroscopically. Ordinarily 1,200 c.c. of the barium enema at 100 F. will suffice to fill the colon. It is important that a uniform technic be followed. I insist on seeing, by means of the fluoroscope, that the cecum is well filled. Massage of the abdomen over the shadow of the cecum is practiced in the anti peristaltic direction. Still further to insure complete filling of the cecum, the patient is sometimes asked to lie on the right side for ten or fifteen minutes after the injection of bismuth, and a second examination is made when the patient returns to the table after evacuating the colon. The roentgenograms are made with the patient lying prone, plate anterior, rather than lying supine, plate anterior. In the latter position "saddling" of the ileum over the iliopectineal line may lead to con-

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fusion. In marked cases of ileocolic valve insufficiency, however, there is never any difficulty in recognizing the terminal ileum.

4. When the valve is once found incompetent it has been possible to demonstrate incompetency during succeeding tests. I have tested the valve a second time in more than fifty cases and on numerous occasions, a third time. The incompetency is seen to be a constant phenomenon in all cases in which it is once seen.

5. In preparation for the enema test, thorough preliminary cleansing bowel-flushings are ordered two hours before the bismuth test. Every once in a while, a patient complains bitterly that the cleansing enemas make her ill. Patients find difficulty in describing their feelings accurately; it seems to be a peculiar, sickening sensation accompanied by abdominal distress and nausea. Almost invariably it is found in these cases that the bismuth enema ascends a considerable distance into the terminal ileum, which seems to be wider than usual.

6. A still more convincing evidence of ileocecal valve incompetency, at least to me, is the reflux of ingested bismuth from the colon into the ileum. I have observed this reflux of ingested bismuth in more than seventy cases. For example, the terminal ileum is seen to be empty at the tenth or twelfth hour following the bismuth meal but at the twenty-sixth hour the terminal ileum is again filled with bismuth though no bismuth has been taken in the interim. Roentgenoscopic examination for this purpose must be conducted with the patient horizontal, so that accurate visualized palpation may be practiced under the fluorescent screen. Often, by massage in the anti-peristaltic direction, bismuth ingesta may be forced back into the ileum from the cecum. Such cases invariably show extreme incompetency to the enema.

7. The literature concerning rectal alimentation contains the statement that if the nutriment be given

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in saline solution it will be more likely to pass the ileocecal valve. In order to test the possible influence of saline solution in testing the ileocecal valve by means of the bismuth enema in a series of fifty colon injections, I added enough salt to the clysma to equal normal saline solution. Comparing this series of cases with the fifty preceding and the fifty succeeding cases in which colon injections were made without salt, there was no difference in the frequency of ileocolic valve incompetencies. Several experiments were made in testing the influence of the composition of the enema. It was found that when barium sulphate, bismuth oxychlorid or bismuth subcarbonate was used in a gummy suspension in water, with or without saline solution, and in buttermilk suspension with or without the addition of salt, a constant proportion of cases, about 1 in 6, of ileocecal valve incompetency, were observed.

It seems to be a current opinion that rectal alimentation is, on the whole, unsatisfactory, although there are enough cases reported of successful rectal alimentation to warrant the continuance of the practice. In the light of our present knowledge concerning ileocecal valve incompetency, it seems to me reasonable to presume that those cases in which rectal alimentation has proved successful are instances of ileocecal valve incompetency.

8. To me one of the strongest evidences in favor of the normal competency of the ileocolic valve is the fact that when it is incompetent, the competency may be restored by a simple surgical procedure. Kellogg, agreeing with me that the ileocolic valve incompetency must be an important factor in the production of ileac stasis, devised a simple but successful operation for restoring the competency of the ileocolic valve. I have already reported postoperative studies on more than one hundred cases in which this operation for restoration of the ileocolic valve competency has been performed. With only three or four exceptions, the Roentgen-ray diagnosis of ileocolic valve

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incompetency was confirmed at operation, and the valve competency was restored by the simple operation above mentioned. That the competency was restored was proved, not only by testing the valve *in situ* before the abdomen was closed, but later by roentgenologic tests. Although, in several instances, more than a year and a half had elapsed since the operation was performed, in only three or four cases out of the hundred did subsequent roentgenoscopy find the valve incompetent.

The effect of the operation on the emptying-time of the ileum has also been tested by the bismuth meal, but thus far no evidence has been secured which would lead me to believe that the operation has caused the slightest interference with the passage of ingested food from the ileum into the colon. On the contrary, the emptying-time of the ileum, which before the operation was in every single instance prolonged, has been definitely shortened, nearly always within eight hours. Emphasis should be laid on the fact that the operation for repair of the incompetent valve has been done, in by far the majority of cases referred to, in connection with some other operation which required opening of the abdominal cavity.

In a few instances in which the ileocolic valve had appeared to be competent to the enema, at subsequent operation the competency of the valve appeared to be open to question, at least in relation to gas, since slight pressure on the cecum was found sufficient to cause a reflux into the ileum after the latter had been thoroughly emptied. In accounting for these cases, I have borne in mind the experimental evidence submitted by Ziemssen, who found that, under deep chloroform anesthesia, the resistance of the valve to reflux from the colon was decreased and fluid could be forced into the small intestine. I have, therefore, assumed that, in these cases, the valve itself was really incompetent, even at the time of the enema; but with the patient awake and the bowel tone normal, the activity of the sphincteric mechanism was sufficient

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to prevent a reflux from the cecum into the small intestine, even though the valve lips did not closely approximate. Further, at operation, the test of the valve competency is usually made with gas and not fluid. The experiments of Senn and others would indicate that one can overcome the competency of the ileocolic valve more easily with air than with fluid, especially when the patient is anesthetized.

9. Another significant observation is made in connection with surgical operation in cases in which ileocolic valve incompetency has been demonstrated roentgenologically. At operation the surgeon often finds the small bowel filled with gas to a very disturbing degree in spite of the careful preoperative preparation, whereas in cases in which the valve is competent the small intestine is collapsed.

10. Still another evidence of normal valve competency is found in the fact that it is possible in the operation of ileosigmoidostomy to construct an artificial ileocolic valve which will stand the test of postoperative roentgenologic studies. The operation does not differ from the ordinary end-to-side anastomosis except that there is a distance of 2 or 3 cm. measured along the surface of the ileum, between the inner and the outer suture-lines. The result is an inversion of the ileum into the colon for about half an inch, forming a circular valve-like fold, which acts as an efficient valve. I have made such postoperative studies in twelve patients thus operated on by Kellogg in which roentgenoscopic tests have proved the competency of the artificial ileocolic valve.

11. At operation, cases of ileocecal valve incompetency show definite deviations from the normal anatomic structure. The crease marking the normal invagination is obliterated; the ileum is pulled out of its cecal pocket; the last inch or two is dilated into a trumpet-shaped sac, which Kellogg has found at operation to have a diameter of from 5 to 7 cm. instead of the normal 3 cm. Kellogg has also called

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attention to a thinning of the walls of the terminal ileum often seen in marked cases of stasis.

12. Post-mortem studies show a great predominance of competent valves. Surely the only function persisting in the cadaver is a mechanical action, yet Kraus in one hundred and fifty necropsies after death from various causes, found the ileocolic valve competent in all but twelve. The reports of a few other pathologists who have published their observations, agree closely with this statement.

In the foregoing pages evidence is cited which, it seems to me, demonstrates the fact that the ileocecal valve is normally competent and that the competency is due to an action of the valve lips which is largely, if not entirely, mechanical. This evidence may be summarized as follows:

1. The ileocecal valve is almost universally present in vertebrate animals; and, at least, in the dog, pig, and cat, the valve is competent to the enema, withstanding enormous distention of the colon by fluid and gas.

2. By means of a string passed through the alimentary canal traction may be made on the valve lips, producing temporary incompetency.

3. In about one-sixth of three thousand persons, most of them constipated and all suffering from gastro-intestinal disturbances, the bismuth enema passed the ileocecal valve and filled the terminal ileum for varying distances.

4. The valve incompetency thus determined is a constant phenomenon in those cases.

5. Patients with incompetency of the ileocecal valve describe characteristic disagreeable symptoms apparently due to passage of the enema into the small intestine.

6. In the marked cases there is also observed a reflux of ingested bismuth from the colon back into the ileum.

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7. The occurrence of the incompetency is, to a large degree at least, independent of the temperature or composition of the opaque enema.

8. The incompetent ileocolic valve may be restored to competency by a simple surgical procedure, the competency persisting in some cases at least a year and a half.

9. In operation on patients with incompetent ileocolic valve the small bowel is found filled with gas to a very disturbing degree.

10. It is possible in the operation of ileosigmoidostomy to construct an efficient artificial ileocolic valve which will successfully act as a barrier against reflux from the colon.

11. Definite deviations from the normai anatomic structure are found at operation on cases of ileocecal valve incompetency.

12. Post-mortem studies show the ileocolic valve to be competent in the great majority of cases.

ABSTRACT OF DISCUSSION

Dr. Lewis Gregory Cole, New York: In 1902 I first recognized a case of ileocecal insufficiency. Since then I have observed it in about the same proportion of cases that Dr. Case has reported. It occurs in varying degrees. In some cases it will only ascend about 6 inches, and in others will extend all the way through the intestines to the pyloric sphincter. Therefore, there can be no doubt that ileocecal insufficiency occurs frequently. The important question is whether or not it is pathologic. I have tried all kinds of arguments to interest surgeons and physicians in this subject, but the most effective is to ask whether or not they would expect symptoms if they were habitually fed the contents of their colon, even though it were administered through a stomach-tube. When the putrid feces from the colon pass into the small intestine, which is relatively sterile and composed of a highly absorbing membrane, one would certainly expect symptoms.

Dr. Walter B. Cannon, Boston: Dr. Case's work has interested me particularly because of earlier work which was done on lower animals. Attempts were made repeatedly in animals to press the material back into the small intestine from the colon, and in spite of repeated pressure it was impossible to cause this material to return to the small intestine once it had got through the ileocolic valve. Nevertheless, in a paper on some work done in 1901, it was shown that

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material injected into the colon might go back into the small intestine, and roentgenograms were taken at that time showing material, introduced into the large intestine through the rectum, that had gone back and filled a considerable part of the small intestine. The most significant part of the work which Dr. Case has reported was that in which he noted that material which had normally passed from the small intestine into the large, so that the small intestine was quite free, was found later back in the small intestine. This observation seems to offer strong opposition to the argument that material coming normally through the small intestine into the colon is different from that introduced per rectum and, therefore, is treated by the ileocolic valve differently from injected material. The mechanism of the return to the small intestine is, I think, a rather interesting one. In lower animals there is naturally a backward movement by the reversed peristalsis or anastalsis of the colon. Does Dr. Case know the mode of this return in human beings? Of course there are well-known instances of attempts by surgeons to keep material from escaping through an opening in the cecum, by uniting the small intestine with the distal colon. The material continued to escape, thus indicating there may be a reverse movement of material through the large intestine. It seems possible, therefore, that sufficient pressure might be developed in the large intestine to carry this material back through the many loops of the small intestine.

Prof. A. J. Carlson, Chicago: There are severe symptoms that we would expect to follow the injection of fecal matter into the stomach: Cats' or dogs' feces diluted to pass through a stomach-tube, of which material 100 c.c. was given each day or every other day for two or three months, has produced no results whatsoever in any of our cats as evidenced in the general metabolism or general behavior of the animals. Does this mean that the same result would be obtained in man? It is possible that fecal material coming into the small intestine from the large intestine without passing the stomach is more toxic than if introduced by the stomach.

Dr. Allan C. Eustis, New Orleans: It is generally accepted that feces or even their extract are highly toxic when introduced into the body. Some stools are more toxic than others, depending on flora and mediums, and the body has the power of breaking up these poisons and rendering them inert. The liver is especially active in this respect, but the heart-muscle, uterus and even subcutaneous tissues also possess this power. Dale has shown that if parahydroxyphenylethylamin is perfused through the liver of a dog, it is broken up and phenylacetic acid is formed. This amin, which is formed by the putrefaction of tyrosin, is capable of raising systolic blood-pressure from 110 to 260, when introduced into

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the vein of a dog, while a like action can be demonstrated in man. This protective power of the liver against intestinal poisons explains the apparent inconsistency in the anatomic and clinical findings in many cases of intestinal stasis. Patients with a very heavy indicanuria and evident intestinal stasis may present no subjective symptoms, owing to the fact that the intestinal poisons are detoxicated by the liver; on the other hand, some patients will have marked subjective symptoms with very little demonstrable abnormality of the gastro-intestinal canal. It is the latter class of cases that are most readily handled along dietetic and hygienic lines, and to which I believe most of our cases of bronchial asthma belong. As I have shown in several communications, histidin on putrefaction yields a toxin which is capable of producing violent bronchial spasm, having been first discovered by Dale while seeking the active principle of ergot, and which he identified as beta-imidazolylethylamin. The lethal dose of this amin to a 300 gm. guinea-pig is 0.5 mg., intravenously. I have found that a 1:1000 solution of beta-imidazolylethylamin hydrochlorid, if macerated with the fresh liver of the turkey-buzzard (chosen because of its well-known resistance to putrid meat) and allowed to incubate at 37 degrees C. (98.6 F.) for four hours is non-toxic when introduced intravenously into a guinea-pig. Evidently, there is some ferment in the liver of the turkey-buzzard which is capable of splitting up this amin, for all precautions were taken for thorough asepsis, incubation was conducted under toluene, heat destroys the action and it does not take place in the cold. It may be assumed that the asthmatic individual has lost the power of detoxicating this particular amin, which is amply borne out by my clinical experience. Dr. Case has demonstrated that a patent ileocecal valve will cause intestinal stasis, and I do not think it was his intention to claim this anatomic variation as the cause in all cases, but I wish to impress the importance of considering the physiologic defences of the body to intestinal poisons before resorting to surgery to correct an anatomic abnormality.

Dr. Seymour Basch, New York: Dr. Case in demonstrating his cases made a statement which I wish to controvert, namely, that this condition, unless remedied, is permanent. That is to say, in each patient in whom he finds incompetency of the ileocecal valve there is no spontaneous recovery. A patient was referred to me by a surgeon with a question of the necessity of an operation, because she had signs which pointed to some obstruction at the sigmoid flexure. In the course of our examination we gave the patient a colon enema and found she had a very long intestine low down in the pelvis, but no sign of any obstruction anywhere or ileocecal incompetency. This patient was placed on the usual anticonstipation coarse diet and treatment and massage

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was given the bowels and she has done very well. She has gained, in two years, over 20 pounds, her bowels are regular, and she feels well. In order to complete our study we gave her another colon enema two years later, and found that the muscles of her abdomen were competent to hold up the intestine. Just previous to that, however, I had heard Dr. Case at the County Medical Society in New York, and in going over the plates in my case I found that the second plate showed a marked incompetency of the ileocecal valve according to Dr. Case's outline. In the first plate no such thing appeared, but at the time of the second plate the patient was clinically very much better. We went over the case, but we found it impossible to understand just why a patient should show incompetency when clinically well and none at all during the height of her illness. It shows that we should not hurry our patients into an operation even if we find conditions that apparently point to surgical necessity. We should try common-sense measures first.

Dr. E. C. Rosenow, Chicago: Did any patients in whom Dr. Case noted incompetency of the ileocecal valve have arthritis deformans?

Dr. George E. Pfahler, Philadelphia: We have seen a number of cases in which arthritis deformans is associated with ileocecal valve incompetency.

Dr. Thomas A. Groover, Washington: Has Dr. Case any theories as to the cause of this condition? Is it congenital or acquired? Has he made any studies of infants to see if the condition is present in infancy? A large proportion of the cases which have presented this condition, in my experience, have been in those persons who have been in the habit of repeatedly taking large enemas with a great deal of pressure. Has he anything to say about this as a causative factor?

Dr. J. T. Case, Battle Creek, Mich.: The pathologic significance of ileac stasis due to ileocecal-valve incompetency I did not attempt to discuss at length here. I tried to confine my remarks to the one anatomic and physiologic question, whether or not the normal ileocecal valve was an efficient barrier against reflux from the ileum. The pathologic significance of ileal stasis is an intricate problem and many phases of it have been touched on. Without discussing whether or not indicanuria is a definite evidence of intestinal stasis, I may state that I have tabulated one hundred cases in which the ileocolic valve was found competent, and another hundred cases in which it was incompetent; one could see at a glance at the indicanuria values, which cases presented the condition under consideration. The patients with incompetent valve presented indicanuria in the majority of cases, whereas the patients in whom the

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valve was competent rarely showed a marked indicanuria. What I have tried to bring out is that the ileocecal valve can be rendered competent on the operating-table. An efficient competent valve can be made in ten minutes by the surgeon, and I consider that this fact tends to prove that the valve is normally competent. As Dr. Cannon has mentioned, one of the most convincing arguments as to the normal competency of the ileocecal valve is the fact that in a large number of cases I have observed a reflux of ingested food from the colon into the ileum. Antiperistalsis surely is an important factor in the production of stasis in the ileum, through incompetency of the valve. It is well known through Cannon's work that, in cases of obstruction in the distal colon, there is an exaggeration of the normal antiperistaltic influence in the proximal colon. This is exemplified in gangrene of the cecum recently seen in a case of malignant obstruction of the sigmoid colon. Exaggerated antiperistalsis is also seen very commonly associated with spasticity of the left half of the colon, especially the iliac and pelvic colon, with resulting functional hindrance to the onward peristalsis through that portion of the bowel. Most of our cases of ileocecal-valve incompetency have been associated with chronic colonic stasis with spasticity and adhesions of the iliac and pelvic colon—what the Germans have called "spastic obstipation." I was much interested in Dr. Basch's case, and should like to know the details which must have been followed out in order to make sure that the test of the valve competency was a thorough-going one. In all our cases we have insisted on a rigid routine, including careful cleansing of the colon by repeated enemas two or three hours before the barium-enema test; careful massage over the asending colon and cecum in the antiperistaltic direction during the injection of the enema; screen observations during the giving of the enema to make sure the cecum is well filled, and the employment of sufficient pressure to distend the cecum. I have never yet seen a case of incompetency in which the surgeons have not been able to demonstrate the incompetency at operation. I certainly agree most emphatically with what Dr. Basch has said about the importance of a thorough trial of non-surgical measures. Out of five hundred or more cases of incompetency of the ileocolic valve, only about one hundred have been operated on for repair of the valve, and many of these repairs were done in connection with other operations requiring abdominal section. There is a good field for further research in this matter in children's clinics. My observations on children have been limited to relatively few. As to the cases of arthritis, I can agree that I have seen a good many of them in which it seemed that the intestinal stasis was an important factor in the etiology.

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STUDIES CONCERNING DIABETES *

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The work, of which this paper will give a synopsis, is the continuation of an experimental study of diabetes described in detail elsewhere.[1] Certain of the results of the previous investigation, bearing on the present topic, may be summarized under the following four headings:

1. Production of Diabetes: Removal of portions of the dog's pancreas produces a lowering of the sugar tolerance. Removal of nine-tenths of the gland results in severe diabetes. When the remnant is larger (for instance, one-eighth), milder types of diabetes result. The course is chronic, extending over months, and the end fatal. Such animals furnish a closer imitation of human diabetes than is produced by total pancreatectomy.

2. Pathologic Anatomy of Diabetes: In the foregoing procedure, duct communication between the pancreatic remnant and the duodenum is preserved. Therefore the acinar tissue does not atrophy, and in some cases it may hypertrophy. The islands of Langerhans show typical progressive alterations, which may be summarized as vacuolation of cytoplasm and loss of granulation, pyknosis of nuclei, loss of cells, and finally disappearance of islands. By suitable controls the changes were shown to be specific to diabetes. Homans[2] has made a careful histologic study of such

* From the Hospital of The Rockefeller Institute for Medical Research.

1. Allen, Frederick M.: Studies Concerning Glycosuria and Diabetes, Harvard University Press, Cambridge, Mass., 1913.
2. Homans, John: Jour. Med. Research, 1914, xxx, 49.

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changes in the islands of diabetic cats by the aid of the admirable methods of Bensley.

3. Pathologic Physiology of Diabetes: An idea has long existed that the disturbance of carbohydrate metabolism in diabetes might perhaps be explained by the assumption that sugar exists in a combined form in the normal body, but that this combination is lacking in diabetes. In the diuretic behavior of dextrose in diabetic and non-diabetic animals, I found contrasts which were interpreted as evidence in favor of this conception, which was therefore put forward as a hypothesis to explain the various phenomena of diabetes. The combining substance was designated by the figurative title of "amboceptor," to indicate its function as a bond between tissue and sugar. The substance which thus combines with dextrose, or some link in the combination, is supposed to be furnished by the islands of Langerhans. For details concerning the hypothesis and the evidence, reference must be made to the original publication.

4. Prevention or Checking of Diabetes: Under certain conditions the presence or absence of experimental diabetes may be determined by the patency of the pancreatic duct. When the pancreatic remnant is sufficiently small, diabetes results, even if this remnant is cut off from duct communication. But, with a slightly greater size of pancreas-remnant, it is possible under favorable conditions in certain cases to show that diabetes occurs if the duct is patent, but remains absent if the duct is ligated. Particularly in one instance it was shown that a diabetes already begun stopped when the duct was ligated. The period during which diabetes can thus be prevented is necessarily limited, since ligation of the duct produces in the dog gradual atrophy and degeneration of all the elements of the pancreas, and this gives rise after weeks or months to the Sandmeyer type of diabetes.

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PRESENT INVESTIGATION

The present research has advanced along the same lines as the preceding one, and has kept in view the same clinical purpose.

I. PRODUCTION OF EXPERIMENTAL DIABETES

A. By Operation

Of several species of animals tried, the dog and cat are the most convenient and useful. Rapid death from cachexia, which I previously described as an occasional occurrence in dogs, is more frequent in cats. In them diabetes is intense and life short after removal of nine-tenths of the pancreas; but by removal of fractions such as three-fourths, four-fifths and five-sixths of the pancreas, one obtains mild or severe forms of diabetes as desired, and such cats live for weeks or months. In cats, more readily than in dogs, can thus be produced mild, transitory, intermittent forms of glycosuria, doing the animal little or no harm, and determined largely by diet (as milk, or sometimes even the feeding of liver instead of ordinary meat). There is a decided tendency toward recovery, though aggravation can often be produced by continuance of improper diet. This form of diabetes may be compared to those mild, intermittent, relatively benign human cases which some persons have supposed to be not of pancreatic origin.

With respect to dogs, the figures given above concerning size of pancreas-remnants hold good for medium-sized animals, such as were convenient for the purposes of the previous research. Present findings indicate that the size of the pancreas in proportion to body-weight in large and small dogs may vary considerably, and that the size of the pancreas-remnant which permits diabetes may vary correspondingly, though a longer series of observations must be completed before making more precise statements. In cats variations of this character are absent or less marked.

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B. By Diet

The papers of Thiroloix and Jacob, previously reviewed, report success in a line of experiment in which earlier workers failed. They state that some dogs after partial pancreatectomy are free from glycosuria on meat diet, but show glycosuria when carbohydrate is fed; and that continuance of carbohydrate diet finally brings the animals into a state of severe diabetes in which they excrete sugar even on meat diet. The reports of the French authors are brief, and contain no mention of controls or proof that such dogs might not ultimately develop severe diabetes, even without carbohydrate feeding. These may perhaps be the reasons why this discovery has received practically no notice in the literature. Independently of these writers I had made similar observations, and believing them to be of importance, I have repeated and extended the work. Based on the experiments so far conducted, it is possible to make the following statements:

1. After removal of sufficiently large fractions of the pancreas, as above described, dogs develop a severe diabetes, in which they show heavy glycosuria on meat diet and also during considerable periods of fasting. The condition progresses steadily downward to a fatal end.

2. When the remnant of pancreas left *in situ* is slightly larger, a condition may be produced in which the fate depends on the diet. On meat feeding such a dog is free from glycosuria and remains so for months, eating his fill every day and maintaining full health and nutrition, with no sign of downward progress; but subcutaneous tests show that the dextrose tolerance is very low, and bread feeding readily produces glycosuria. A return to meat diet stops the glycosuria; but if the bread diet and accompanying glycosuria are maintained for too long a time, the glycosuria then continues, even on meat feeding. The

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diabetes thus produced is not inferior in severity to that resulting from simple removal of larger fractions of pancreatic tissue, and the downward course and fatal termination are similar.

3. When the pancreas-remnant is still larger, glycosuria is absent on meat diet, and on bread diet may be absent or transitory. Such animals may remain in excellent condition indefinitely on bread diet, free from glycosuria or any downward tendency; but if sufficient sugar is added to the diet, glycosuria can be produced and maintained. After a period of such glycosuria, the animal reaches a condition in which it is glycosuric on bread diet. By prolonging the glycosuria on bread diet, the dog finally reaches the condition of severe diabetes, with glycosuria on meat diet, and continuous downward progress. For such sugar feeding I have ordinarily used commercial glucose. The experiments succeed best in greedy dogs and those naturally fond of sugar. A decided aversion to sugar on the part of the dog may spoil such an attempt through failure of appetite.

4. When the pancreas-remnant is still larger, sugar feeding may produce transitory glycosuria, but it cannot be made to continue. The sugar tolerance is lower than in normal dogs, but nevertheless the doses of sugar necessary to produce glycosuria are higher than can be tolerated as a daily routine by the gastrointestinal canal. Persistence in the attempt to maintain glycosuria causes diarrhea and illness. The dog refuses to eat, sugar given by stomach-tube is vomited, and true diabetes remains absent. Similar results can be obtained with cats by feeding carbohydrate in the form of milk.

The changes previously described as occurring in the islands of Langerhans occur whether the diabetes follows directly on the operation alone, or has been induced in the prepared animal by means of modification of diet. The best of the control animals are still

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alive; but the control tissues thus far examined indicate that in animals predisposed by operation, when diabetes is prevented by carbohydrate-free diet, the signs of exhaustion and degeneration in the islands of Langerhans are absent. If further study shows that this is the case, it will afford additional evidence that these changes have a direct relation to the diabetes and that they are a result of functional overstrain.

While various factors are undoubtedly concerned in the production of human diabetes, clinical observations indicate that diet is an important one. It is generally recognized that there is a higher incidence of diabetes among those races or classes of people who use an excess of carbohydrate. Luxurious living and sedentary life are thought to predispose to this disorder. The experimental observations on animals suggest an explanation of this relationship between diet and diabetes in the human subject. If individuals differ in the strength of the pancreatic function as in other functions, in some this may be so weak that diabetes comes on in early life irrespective of the diet. In others this function may be only a little stronger, so that diabetes may be delayed until later in life or even to the period of senility, when there occurs an impairment of various functions. Others may be more or less below the average in pancreatic functional power, but under ordinary circumstances this is sufficient to prevent the occurrence of diabetes. In these persons, however, who might otherwise go through life with no sign of diabetes, an excess of starch in the diet may serve as an exciting cause of diabetes, and the degree of such excess may help to determine the earlier or later onset and the milder or severer type of the disorder. In other persons of this sort, as in the corresponding type of dogs, the pancreatic function is able to deal safely with as much starch as can be digested, but a sufficient excess of sugar is an effectual cause of diabetes.

It is impossible to produce diabetes in the normal dog by an excess of carbohydrate feeding, since if too

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much sweet or starchy food is taken, indigestion results and automatically stops the ingestion. But in dogs after operation and in predisposed human patients, pancreatic weakness may reverse the normal relation, so that the organism can digest and absorb more carbohydrate than it can combine and assimilate; and in this condition the production of diabetes by improper diet is possible. The question of diabetes may then be regarded as a balance between the digestive and assimilative functions. Moreover, the body is equipped with no natural safeguards against this unnatural condition. There is no reflex or other mechanism which points plainly to the cause of the trouble and compels its cessation. On the contrary, the impaired nutrition due to failure of assimilation affects the organism as if it were due to deprivation of food. The response commonly shows itself in the harmful form of increased appetite. The taking of excessive food injures the assimilation still further, and thus a vicious circle results.

II. PREVENTION OR CHECKING OF EXPERIMENTAL DIABETES

The therapeutic experiments in animals pertain to two groups of cases, those in which diabetes is produced by simple operation, and those in which diabetes is produced by diet in predisposed animals.

1. Animals made diabetic by operation. In animals from which an excessive amount of pancreatic tissue is removed, a diabetes may be obtained which is so severe that fasting will not produce sugar-freedom. But when the pancreas remnant is of suitable size, for example, one-tenth, though the resulting diabetes is permanent on meat diet and will end fatally if allowed to persist, nevertheless a few days of fasting at the outset will produce sugar-freedom. If the diabetes is allowed to continue longer, a much longer period of fasting may be necessary for sugar-freedom, or it may be impossible to obtain. If, after obtaining

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sugar-freedom, feeding of protein and fat (with occasional bones) is begun very cautiously, in quantity only enough to maintain the animal in its thin condition, such dogs remain free from diabetes. The longest experiment to date is that of a dog which, possessing less than one-tenth of the pancreas, has been kept free from diabetes for six months, and there is at present no indication that the condition cannot be continued indefinitely. If an attempt is made to increase the weight of such an animal, glycosuria soon appears and must be checked by renewed fasting. Such dogs, though very thin, are vigorous and lively. They contrast sharply with dogs which, after similar operations, are allowed to remain diabetic on full meat diet. Though the latter animals at first appear much better nourished, they finally emaciate in spite of the most enormous eating; and when they have become as thin as the rogs above described, weakness and cachexia are very evident, and the progress continues downward to death.

2. Animals made diabetic by diet. When the animal possesses perhaps an eighth or a sixth of the pancreas, and is not diabetic after operation but is then made diabetic by excess of carbohydrate, this diabetes at first can be stopped by a simple change to meat diet. After a longer duration, the diabetes no longer stops on carbohydrate-free diet; but after a period of fasting, the time depending on the severity of the diabetes, the urine becomes sugar-free. With still greater duration or severity of the diabetes, sugar-freedom is no longer obtainable by fasting. When such animals are made sugar-free, they may be kept so by the same procedure as described for those of the former group. When the diabetes is thus checked fairly early, it is evident that the prognosis is much more favorable than in animals of the previous group, for in the present animals the removal of pancreatic tissue was considerably less, and the cause of the diabetes is partly a functional change. Accordingly, it is found that the

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diet can be gradually increased, and in favorable cases the animals be brought to a higher level of weight and nutrition than is ever possible with dogs of the preceding type. Up to the present, however, the experience has been that these dogs are never able to return quite to the condition which they enjoyed before the diabetes, and which is permanently maintained by similar dogs in which diabetes is prevented by suitably chosen diet from the outset. As above mentioned, dogs so treated can be kept indefinitely at full weight and well-being when placed after operation on a diet which keeps them free from glycosuria. But when diabetes gravis has been produced and allowed to continue long enough to demonstrate its reality, while it has been possible by the foregoing method to stop the diabetes and bring a dog back to within a kilogram of its normal weight, every attempt to produce a further gain in weight has brought a return of glycosuria, which must be checked by fasting. Such dogs, however, are so near to normal that, if they are mixed up in a yard with ordinary dogs, a stranger might be unable to tell one from the other.

The microscopic examinations of the pancreas remnants of such animals as have yet come to necropsy from both of these groups show that in both alike the island cells are well endowed with cytoplasm and granules, and degenerative processes are not seen; but there is a decided impression that the islands are inferior in both size and number to those of normal animals. If this impression proves valid in further experiments, a correlation readily suggests itself between the observed anatomic change and the diminished pancreatic function described in the preceding paragraph.

In my previous report I have shown that by removal of suitable fractions of the pancreas, animals may be brought so close to the verge of diabetes that the removal of less than a gram of additional pancreatic tissue suffices to bring on diabetes. According to the

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"amboceptor" hypothesis, the pancreas furnishes some definite substance or substances used by the cells of the body for the metabolism of carbohydrates and perhaps also of other foods, the "amboceptor" being used up in metabolism and bearing some quantitative relationship to the metabolism. Extending this theory to the foregoing experiment, one may say that the fragment of pancreas which barely prevents diabetes is the smallest fragment which can supply the minimum quantity of "amboceptor" necessary for the animal's metabolism. When such a pancreatic fragment is even slightly reduced, the supply becomes slightly deficient in quantity, and diabetes accordingly begins and runs the usual downward course.

Present observations prove that the reverse of this experiment is likewise possible, that is, when an animal and its food consumption are suitably reduced, a pancreatic remnant otherwise inadequate becomes adequate; diabetes under these conditions may be prevented or be checked after it has appeared. Prevention or cessation of diabetes previously observed in consequence of ligature of the pancreatic duct may be explained by the impaired food absorption and chronic malnutrition of the animal. Similarly in dogs which develop distemper after the pancreatic operation, and which therefore refuse food and emaciate rapidly, diabetes may fail to appear or may stop after it has appeared. Interpreting these experiments on the basis of the "amboceptor" hypothesis, one may say that an otherwise insufficient amount of "amboceptor " may become sufficient when the metabolism of the body is artificially diminished.

III. CLINICAL TREATMENT OF DIABETES

In the clinical literature of diabetes there are authentic reports of certain cases in which even severe diabetes has cleared up spontaneously and completely on the onset of cirrhosis of the liver, cancer, tuberculosis or some other wasting condition. Other cases have

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notably improved. Probably the most severe and certainly the best studied of such examples was presented by one of Joslin's patients,[3] in whom a long-standing and dangerous acidosis disappeared entirely, and a negative carbohydrate balance became positive, following the onset of tuberculosis. When these cases are considered in the light of the experimental study of animals, the possibility is suggested that we have an indication for a rational method of dealing with diabetes, or impaired pancreatic function associated with overstrain.

Notice should be taken of the advantage possessed by most diabetic human patients over diabetic dogs. Even the most hopeful of the above-described dogs has only a small fraction of the pancreas. Most of the organ is hopelessly gone, and the presence or absence of diabetes is determined by functional influences acting on the little remnant. Furthermore, even when the diabetes is inaugurated by functional means, the organic degenerative changes quickly ensue in the islands of Langerhans. Under these conditions it is surprising that results are obtainable by treatment at all. On the contrary, in typical human diabetes the entire pancreas is present, and there are indications that functional disturbance is an important factor.

It has been thought justifiable, therefore, to undertake the treatment of a limited number of patients by a method based on the principles derived from the experimental work as indicated in this communication. The number of patients so far treated is limited, but the results obtained indicate that the same method employed in rendering the diabetic dog free of glycosuria and prolonging its life is efficacious in eliminating glycosuria and acidosis in the human patient. To what extent life may be prolonged by this method only a large statistical study will show. The observations

3. Benedict, Francis G. and Joslin, Elliott P.: A Study of Metabolism in Severe Diabetes, Carnegie Institution of Washington, 1912, Case R, p. 55.

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so far indicate that the method is not harmful, and when carried out carefully seems definitely beneficial.

The method of treatment is in brief as follows: If the patient is moderately emaciated, with a negative carbohydrate balance and acidosis, he is put to bed and receives no food whatever. If coma seems imminent the usual emergency treatment with purging, stimulants, alkalies and large amounts of water should, of course, be carried out. In addition to fasting, alcohol is important in the treatment at this stage. From 50 to 250 c.c. of whisky or brandy may be given in each twenty-four hours in small doses, from 10 to 20 c.c. every one to three hours during the twenty-four. As soon as the glycosuria stops and the acidosis diminishes, which even in severe cases may be within forty-eight to ninety-six hours, the amount of alcohol and alkali may be reduced. Fasting and moderate dosage of alcohol are continued for from twenty-four to forty-eight hours longer, however, depending on the patient's strength. The alkali is now stopped, and feeding with starch is commenced in order to clear up the last traces of ketonuria. The kind of starch is of minor importance. Green vegetables are useful because their carbohydrate and food value is so low that they can be given in considerable bulk, and this bulk is agreeable to the patient for relieving his feeling of emptiness. Neither fat nor protein is added. For the first day, the food is chosen to represent a carbohydrate content of from 10 to 40 gm. This is divided into four to ten equal portions and fed at equal intervals during the day. If glycosuria remains absent, the ration for the next day is doubled, to represent 20 to 80 gm. of carbohydrate, similarly divided into numerous small portions. On the next day it is sometimes possible to increase the ration to 100 gm. of carbohydrate, without glycosuria. About this time, especially if glycosuria has appeared, another fast-day is interposed, from 50 to 200 c.c. of whisky being given. Present experience indicates that

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even in severe cases ketonuria may by this method be made to disappear entirely. Several repetitions of the foregoing routine may be necessary for this purpose. All food contains danger, tending toward either glycosuria or ketonuria. The carbohydrate of the diet is seldom reduced below 50 gm., and is preferably kept higher. If carbohydrate must be kept low, the total diet is kept low. The diet is so chosen that glycosuria, not ketonuria, is the signal of overstrain. Fasting-alcohol days are given not merely whenever this signal appears, but also at close enough intervals to prevent it from appearing, even every two or three days if necessary. If there has been no glycosuria, a slight addition to the diet is made after each fast-day. Each day's diet is calculated exactly, and the nitrogen-balance is watched. It is thought that no matter how low the assimilative power, the attempt to feed in excess of this power is harmful, and it is possible that by rest the assimilative function may gradually become stronger. With improvement in the patient's condition, the carbohydrate in the diet is further increased. Increase in weight, however, is not attempted at this time. From our present point of view, contrary to the generally held opinions, the attempt to increase weight should be the last rather than the first step in treatment. It is attempted to keep the metabolism at the lowest safe level until the patient is taking from 100 to 150 gm. of carbohydrate (mostly as green vegetables) daily, with fast-days interposed often enough to prevent any trace of glycosuria from appearing. Then protein is cautiously added, always being kept rather low; and in favorable cases the weight and well-being may finally improve under gradual additions of fat.

The radical procedure here described is that used for the most severe cases. In milder cases the treatment may be correspondingly milder. Primary loss of weight is intentional. The purpose of the treatment is not to confer temporary comfort or appearance

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of well-being, though various symptoms, including polyphagia and asthenia, may actually be relieved When there is extreme cachexia and emaciation, the difficulty is greatest. It may then be necessary to juggle very carefully the three factors of glycosuria, acidosis, and nutrition.

A report giving the results of this form of treatment in a series of cases will be published later. It is felt that the conception underlying this method of treatment, based on experimental observations, is new, though certain details have long been recognized as of importance. According to this method, alkali treatment is not employed, unless for a brief period at the outset, while severe acidosis is being combated. The alkali treatment has been called the most brilliant discovery in the modern study of diabetic therapy. It is indeed a valuable means for facilitating the excretion of acetone bodies. But under an efficient treatment of diabetes, acetone bodies should not be excreted. They should be burned.

Though it seems possible thus to check all active symptoms, with apparent benefit, even in very severe cases, yet it is felt that the ultimate outlook for these patients is far less favorable than it would be if they could be treated earlier. The best therapeutic hope is believed to lie in the application of this principle of treatment at the earliest possible stage in diabetes.

ABSTRACT OF DISCUSSION

Prof. A. J. Carlson, Chicago: Does Dr. Allen consider the evidence conclusive that mere obstruction of the pancreatic duct in dogs leads to the atrophy of all the elements in the pancreas? In the rabbit this is not the case. I have seen in dogs, in connection with other work, the islands persist for six or nine months, that is, enough of the island tissue to prevent diabetes.

Dr. Allan C. Eustis, New Orleans: Has Dr. Allen noticed the appearance of the acetone bodies in the urine of these dogs; whether they were present during the fasting stages and how soon they would appear; and also whether

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the amount of the pancreas left in had anything to do with the amount of rapidity with which the acetone bodies appeared?

Dr. F. M. Allen, New York: Answering the latter question first, I have never seen a satisfactory reproduction of human acidosis in dogs. A few authors have reported considerable acidosis in totally depancreatized dogs, and even death in coma, but these cases are the exception rather than the rule. The difference between carnivorous animals and man as respects acidosis is one reason for attempting to reproduce diabetes in other species which are more like the human in this respect. With regard to Dr. Carlson's question, two factors must be considered; one is species, and the other is time. In rabbits and guinea-pigs, following ligature of the pancreatic duct, authors have proved that numerous normal-appearing islands of Langerhans are present long after the disappearance of all acinar tissue. The pancreas is described as being replaced by fibrofatty tissue and though the islands pass through phases of degeneration and regeneration, the island tissue is not permanently destroyed but apparently persists indefinitely. Such animals are free from diabetes, but it has never been proved that true diabetes can be produced in these species by any means. In dogs, on the other hand, ligature of the pancreatic duct is followed by scar-tissue formation, and islands as well as acini are destroyed. Such degeneration of a ligated pancreas-remnant gives rise to the Sandmeyer type of diabetes. In the dense fibrous tissue at the site of the pancreas in dogs dead from prolonged Sandmeyer diabetes, it is said that neither acini nor islands have been found. The time-element is involved in experiments such as Macallum's, which undertake to show that islands persist at a time when all acini have disappeared, and that the dog at this time is not diabetic. Positive results in such experiments may furnish evidence in favor of the insular hypothesis; nevertheless after a longer time-interval the islands may be expected to degenerate and disappear. Indefinite persistence of islands in the ligated pancreas, as proved for the rabbit, has never been shown in the dog.

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ABDERHALDEN'S TEST IN THE DIAGNOSIS OF CANCER

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Statistics, together with the dreadful nature of the disease, demand that any and every method proposed for the early diagnosis of cancer should be thoroughly and impartially tested. Emil Abderhalden of Halle, Germany, recently presented to the medical profession a biologic test that is demanding the closest attention. Time alone can thoroughly prove the accuracy of his test, and only as impartial reports of large numbers of cases examined are offered by reliable workers. It is readily apparent by a casual review of the literature pertaining to the test that the differences in the results obtained are due to some variation as to technic, selection of tissues used as fundaments, unless the test is unreliable. Frankel states that the Abderhalden reaction was not reliable in the cancer cases examined at the research institute at Heidelberg, while Frank and Heimann, Marcus, Epstein, Erpicum abroad, and Lowry in this country report more favorable results.

Apparent errors in the specificity of the test do not appear to be evidenced by malignant conditions failing to give a reaction, as much as the finding of a positive reaction in individuals almost certainly not malignant. My experience with this test would indicate that there are some conditions clinically not malignant that occasionally give a positive malignant reaction when not expected; as, for instance, certain pathologic conditions, as myomas of the uterus, and even the physiologic condition of pregnancy in a few instances, as has also been noted by Frank and Hei-

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mann. Especially is such a reaction to be obtained if a cancer of the uterus were used as the prime fundament. The uterine cell in such tissue is digested instead of the cancer-cell. For this reason a cancer of the uterus should never be used for making the test. Data based on such work are necessarily unreliable (Table 1). The majority of positive reactions tabulated as errors in my clinically non-malignant cases were secured by the use of such tissue, used for a time without recognizing its treacherous nature. It is notable that all of these cases possessed a distinct pathologic uterine condition—myomas, metritis and endometritis. The elimination of these cases in my series greatly increased the percentage of accurate interpretations, in nearly one hundred examinations, largely clinical as well as experimental. There were, however, a few with which by repeated examinations with indifferent cancer tissue only a positive reaction could be obtained. Of these one has to class them either as errors, or admit the possibility of an individual giving the test that has harbored an invasion and recently dispelled it (Vaughan), or possesses an active invasion (second-degree cell proliferation of Mayo) that had not at the time of the test made itself manifest by even suspiciously clinical symptoms. I am led to consider the latter possibility by the developing histories obtained in several of these cases since the making of the tests. That is, they have progressed to a third-degree proliferation. I admit that in the remaining few cases there is absolutely no reason as yet for thinking the test other than erroneous.

PRACTICAL APPLICATION

The differences of opinion that arise relative to the findings in the suspiciously malignant cases develop the practical application of the test. The finding of a positive reaction in a known malignant condition is gratifying but of little practical value to the sufferer. The weight of responsibility resting on a positive or negative finding in suspiciously malignant individuals

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is tremendous. Until further work perfects the technic, a clean-cut, negative reaction carries much more

I believe that the Abderhalden reaction is especially valuable in answering quite positively any question

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arising as to the actual cause producing some of the border-line roentgenographic filling defects. The

tion of actual cancer-cells than any other form of tissue available.

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Fresh tissue secured from a person accidentally killed should always have first choice. Tissue from the operating-table usually has to be accepted and is far preferable to tissue obtained at necropsy. Tissue received at necropsy that requires many repeated boilings to render it negative to ninhydrin should be condemned. When tissue requires many repeated boilings, there is such an amount of the protein material already broken down, at or near the amino-acid stage by decomposition, that it reacts to ninhydrin even though it may not have been acted on in the incubator by its accompanying serum. Bad tissue explains practically all of our erroneous findings. Bad thimbles come second.

Tissues used as substrate or fundament should be accompanied by a carefully prepared statement concerning the history of its donor, together with a microscopic study. Series of cases reported should be accompanied by a detailed description of the tissues used as fundaments. It will be evident from such data that there is a great difference as to the reliability of different tissues. It will also materially aid in standardizing the technic and value of the test.

Providence determined circumstances leading to the possession of the first cancer-tissue used in my research work. The results obtained with this tissue were very gratifying, and the reactions were cleaner-cut, giving a distinctly lower percentage of possible errors than some tissue I have been obliged to use since. This tissue was a lymphosarcoma of the cervical and retroperitoneal glands. The all-cancerous nature of this material determined its value. A careful study of the accompanying tables will readily demonstrate the most desirable histopathologic requirements of the prime fundament. Combinations between prime and secondary fundaments suggest the possibility of being able to roughly locate a malignant invasion

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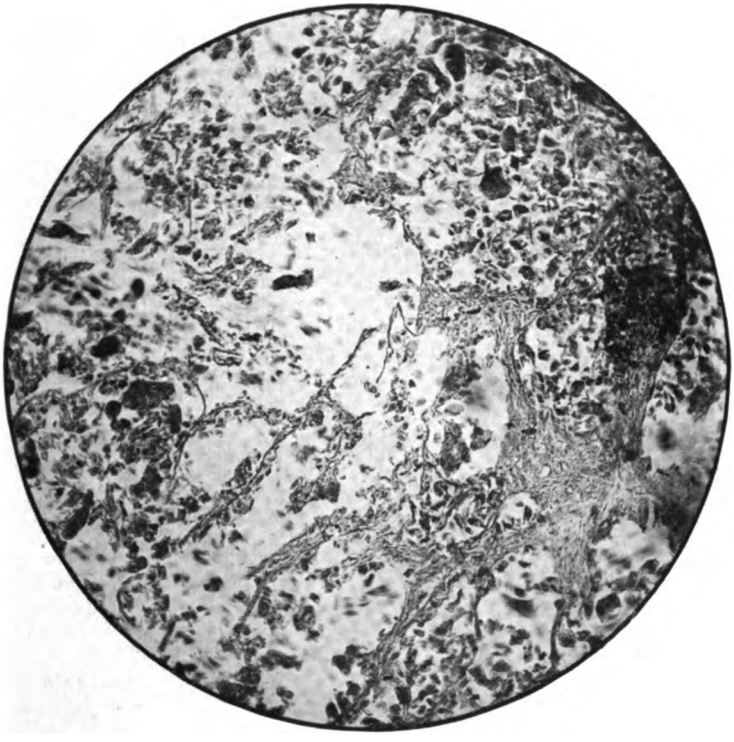

Fig. 1.—Cancer nodule of the liver.

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Fig. 2.—Cancer of the uterus.

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Fig. 3.—Cancer of the testicle.

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Fig. 4.—Adenocarcinoma of breast.

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Fig. 4.—Adenocarcinoma of breast.

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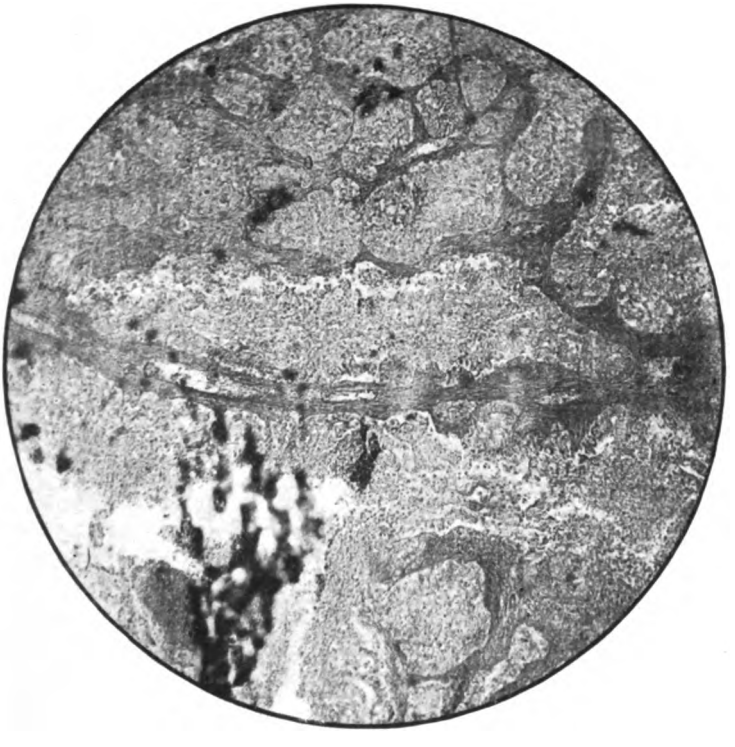

Fig. 5.—Sarcoma of the retroperitoneal lymph-glands.

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(Lindig, Kabanow). Varying the combination formulas of fundaments in reexaminations is of distinct advantage in suspiciously malignant investigations (Table 3).

I fully realize the fact that the histories tabulated as confirming the accuracy of the test are in some ways possibly unreliable. I have, however, endeavored to so use only such cases as gave very definite clinical manifestations, or those in which the cause of death was due unmistakably to the effects of a malignant disease. I readily appreciate that the only absolute solution of the problem is the number of malignant cases examined that give a positive reaction in which a microscopic diagnosis is able to verify the validity of the reaction. There will be, however, a border-line class of cases giving the reaction in which pathologists may differ, owing to the presence of a positive reaction in very early malignant cases, microscopically not well defined.

SUMMARY AND CONCLUSIONS

1. Keep carefully prepared histories of all cases examined.

2. There should be a follow-up interest, especially in such cases as gave a positive reaction, not clinically malignant at the time of making the test.

3. Care in selection of fundaments should be observed, keeping a carefully prepared statement concerning their donor, with a microscopic study of each.

4. Varied association of data pertaining to patient and fundaments, with reaction obtained is essential.

5. Reexaminations in determining the progress of the disease are of especial value.

6. As yet the test cannot be considered as strictly specific for the diagnosis of cancer.

7. A positive reaction, however, should receive careful consideration before calling it erroneous.

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AUTHOR'S TECHNIC

My technic is given in detail, that the reliability of my work may be judged accordingly.

Only distilled water, distilled at the time of making the test, was used, except in a very few instances in the very first of my work. All glassware is carefully washed, drained, and rinsed with alcohol, followed by ether; it is then sterilized by dry heat. The entire work has been done in a room never used for bacteriopathologic work. All apparatus is reserved exclusively for this work. I have used a few of the 579 Schleicher and Schuell thimbles, but practically all of my work was done using the 579A thimbles, selected as follows:

The thimbles were first well soaked in cold water, then boiled for five minutes. When cool, they were placed on the inner end of the funnel-form stopper of my dialyzing chamber. This was done at first with bare, well-washed hands, but now I use rubber gloves entirely and handle the thimbles as little as possible, rinsing with sterile distilled water before replacing in my dialyzing outfit, previously filled with 15 or 20 c.c. of distilled water. Cotton plugs were placed and then the entire outfit was sterilized by steam heat. No part of the remaining technic allows of the possibility of contamination of the outer wall, or surrounding sterile distilled water. Chloroform and toluene were added through the side tube provided for this purpose. Sufficient toluene was used to overlie the surface thoroughly. The apparatus was then ready to receive the materials intended for dialysis. New thimbles were filled with 5 c.c. of a 1 per cent. silk peptone (Hoechst) solution, covered with 10 drops of toluene, placed in an electrical incubator for from fourteen to sixteen hours. After removal from the incubator, 5 c.c. of the diffusiate were tested with 0.2 c.c. of a 1 per cent. ninhydrin solution. Only those thimbles giving a corresponding *faint* shade of blue color were reserved for further examination.

The reserved thimbles were then run, first with 5 c.c. of a 10 per cent. solution of egg albumin, and if any were found to give the ninhydrin reaction they were immediately discarded. By selecting only such thimbles as give a faint blue ninhydrin reaction in the first test, seldom one is found that is permeable to the albumins. New thimbles are never used in an actual test until they have been run at least twice with human serum and test negative, 5 c.c. of diffusiate to 0.2 c.c. of a 1 per cent. solution of ninhydrin; examination is run in duplicate with a previously tested and satisfactory set of thimbles and the new thimbles judged accordingly.

I have always selected thimbles in groups of four, notching the top with one to four notches, and punching in the side

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just below the notches one or more holes; in any set of four, each has the same hole-punch as one 0 to four 0; or one 000 to four 000. Each set of thimbles is placed in a wide-mouthed container, of 120 c.c. capacity. The bottle was then filled with distilled water and the whole placed in a steam sterilizer for one-half hour. After removal and cooling, 1 c.c. of chloroform was added, and the surface of the chloroform-water was well covered with toluene. The cork stopper was put in place and the thimbles then set away in the ice-box, kept at a temperature of about 8 C. (46.4 F.). Control experiments demonstrate that only the thimbles barely permeable to the silk peptone solution are suitable for the cancer-test as cancer-tissue is quite readily broken down by the "protective ferment" in the dialyzing thimble.

Preparation of tissue, selected as previously suggested, must be complete in the removal of all traces of blood and the saving of only that portion of the cancerous material that contains the smallest amount of near-normal tissue invaded. Such tissue is cut up into small pieces about the size of a pea, well washed, and then thrown into a large volume of boiling water, 10 parts by volume, containing 1 or 2 drops of glacial acetic acid, and allowed to boil for just five minutes. The tissue is then drained of its boiling water and again similarly boiled except that no acetic acid is used. The boiling is repeated in this manner for from three to six boilings, until the boiling-water gives not the slightest reaction with ninhydrin, using 1 c.c. of a 1 per cent. solution of ninhydrin to 5 c.c. of the liquor. Any tissue requiring more than five or six boilings is discarded. After the tissue has tested negative to ninhydrin, by boiling in bulk, the tissue is then placed in small quantities—about 15 gm.—in a 60-c.c. Erlenmeyer flask, and again boiled. After the contents of the flask have cooled, chloroform is added, and the whole covered with toluene, the mouth of the flask is plugged with cotton, and the tissue thus placed in the ice-box in the water in which it was last boiled. Whenever tissue is removed for use, the remaining tissue is similarly treated.

At least 30 c.c. of blood should be taken from the patient, either by venipuncture or actual bloodletting under strict aseptic precautions, and always using only dry glassware. The blood is usually collected by using a modified Jellinghaus-Losee outfit. Constant rigidity should be maintained in using only blood obtained before the morning meal, or if the blood has to be taken in the late afternoon, the patient should present himself without having eaten dinner; not even a lunch is allowed. The blood is allowed to stand at room temperature in the collecting outfit until the serum separates, which varies from three to twelve hours—usually four to six hours sufficing. Accordingly, blood collected at 8 o'clock in the morning is usually ready for setting up in the after-

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noon; that collected in late afternoon is ready next morning, but should be kept in an ice-box over night.

During the time that the blood is separating, apparatus required for the test should be made ready; and the fundaments to be used should be reboiled and made to test negative to ninhydrin, using 1 c.c. of a 1 per cent. solution to 5 c.c. of the boiling water. In reboiling the tissue, care should be taken to use ten times by volume the amount of distilled water as tissue to be prepared.

The separated serum is pipetted off from the collecting outfit into a centrifuge tube, and centrifuged at *high* speed until the serum is perfectly clear, and free from any reddish tint or cloudy appearance. One and one-half c.c. of such serum is placed at once in a small test-tube in the water-bath, maintained at a temperature of 56 C. (132.8 F.), for the purpose of inactivating this amount of serum, which is to be used as a control along with the prime fundament to be used with the untreated serum. At the same time that the serum is being inactivated, the dialyzing outfits as described in a previous article are sterilizing. After the outfit has been sterilized and cooled, chloroform is added through the side tube and then sufficient toluene to cover thoroughly the sterile, distilled water surrounding the sterile thimble free from all possibility of outside contamination due to the special construction of this apparatus. Each dialyzing outfit is labeled and the formula made up of various fundaments to be used, writing on the label the particular fundament and the amount of serum that each outfit is to receive. Then a small piece, from 0.5 to 1 gm., is dropped through the funnel-form stopper supporting the dialyzing thimble on its inner end, in spaced relation to the wall of the outer container. One c.c. of serum is then added to each (formerly used 1.5 c.c.) except to the one to receive the inactivated serum. After placing the serum, 10 drops of toluene are added, replacing the cotton plug, at which time the entire set is ready for the incubator. They are placed in an electrical incubator, at 37° C. (98.6 F.). Abderhalden specifies in the pregnancy test that dialysis should go on for from fourteen to sixteen hours; experience with his technic in the cancer test indicates that a shorter period, about twelve hours, gives better differentiation of color reactions, and so, better satisfaction.

After removal from the incubator, the funnel-form stopper supporting its dialyzing thimble and contents is removed. Five c.c. of the dialysate is placed into a calibrated test-tube, to which is added 0.1 c.c. of a 1 per cent. solution of ninhydrin. The test-tube with its contents is then boiled with its fellow in an electrical heating device so constructed that a definite, constant and predetermined heat is applied, boiling the contents of the test-tubes for exactly one minute (author's electric test-tube heater). By thus boiling the

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dialysates with ninhydrin, the ratio of evaporation is constant for each. After cooling for one-half hour, interpretations are made by the color reactions obtained. If the test has gone on satisfactorily, negative reactions will be apparent by the entire absense of any color in the contents of its test-tube. Positive reactions will manifest themselves by a distinct, usually not too deep, bluish-purple color in the contents of the test-tube. Such reactions are easy of interpretation as to reactions obtained. The association of all the reactions obtained, together with the particular formula for the test, is necessary for the making of a diagnosis. My most frequently used formula for a set of four thimbles is the following:

One thimble containing the prime fundament and untreated serum; one thimble containing the prime fundament and inactivated serum; one thimble with either serum or fundament alone, as might be indicated by a suspicion as to the reliability of either one or the other; the fourth thimble contains the secondary fundament, common to any particular series of studies. Invariably my secondary fundament has been a placental protein. In a few instances I have also used muscle-tissue, brain-cortex, and thyroid gland. When the interpretation of the test depends on the meaning of various shades of color reactions, it cannot be considered in any other way than unsatisfactory, and indicates the necessity for a reexamination. Whenever possible, controls should be run using the same formula either with a known normal or malignant. The larger number of my cases were run with a known control, but this was not invariable.

Gryphon Block.

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A STUDY OF THE FERMENT ACTIVITY OF THE BLOOD-SERUM

DURING PREGNANCY AND UNDER NORMAL AND PATHOLOGIC CONDITIONS *

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CHICAGO

A large amount of work has been done with the Abderhalden test, using the dialysis method. Almost diametrically opposite conclusions have been arrived at by the various workers in this field. L. Flatow,[1] using an acetic acid coagulation method, and neutralizing with calcium oxalate, found that all serum has the power of digesting placenta. H. Kämmerer, M. Clausz and K. Dieterich[2] found that serum of non-pregnant individuals sometimes reacted positively, and that the serum from known pregnant individuals sometimes reacted negatively. Sunde,[3] in eighty-three cases, found that the test corresponded to the clinical diagnosis in seventy-five cases; and that eight non-pregnant cases gave a positive reaction.

Stoekel[4] believes that the proof of the specificity of the reaction is still lacking. He thinks that it, at present, is of little significance for general practice or forensic medicine. A. K. Pain,[5] on the other hand, feels that with good laboratory technic the test is the most accurate of the present-day methods of diagnosis and ranks with the Wassermann and Widal tests.

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* From the Department of Research Medicine, University of Illinois, Chicago.

1. Flatow, L.: München. med. Wchneschr., 1914, lxi, No. 9.
2. Kämmerer, H., Clausz, M., and Dieterich, K.: München. med. Wchnschr., 1914, No. 9.
3. Sunde: Norsk. Mag. f. Laegevidensk., 1913, lxxiv, 1234.
4. Stoekel: München. med. Wchnschr., 1913, No. 9.
5. Pain, A. K.: Boston Med. and Surg. Jour., 1914, clxx, 303.

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Williamson[6] found that in twenty known pregnant cases the reaction was positive in all; while in thirty known non-pregnant cases the results were uniformly negative. In sixteen unknown cases, however, the reaction was wrong in two and doubtful in two. In other words, the reaction was of no value in the diagnosis of 25 per cent. of the unknown cases. Oller and Stephan[7] conclude that, while pregnant serum contains proteolytic ferment that can be demonstrated by the Abderhalden dialysis method, the reaction is of little clinical value, because a relatively large number of non-pregnants likewise give the reaction. They mention Lichtenstein, Rubsamen, A. Mayer, Tschudowsy, Gambaroff, Stange and Aschner as supporting Abderhalden claims, but point out the fact that these investigators rarely show controls with normal serum or, what is of more importance, with serum from pathologic cases. Also that Aschner gets 30.5 per cent. positive reactions in non-pregnant cases. Mühsam, Hies and Lederer, Werner and Wininwarter consider the reaction as entirely untrustworthy. Noncommital are Schafer, Ebeler and Lohnsberg, who report positive reactions in 12.5 per cent. of non-pregnant cases, Frank and Heiman, Frank and Rosenthal, Kohler and Deutsch.

E. Schiff[8] concludes that the test is entirely reliable, but he gives no details as to his technic or controls. He admits getting seven weak positive tests and controls in thirty-six non-pregnant cases. Schwarz[9] found forty-one known pregnants who gave a positive test, while thirty known non-pregnant cases gave a negative reaction. The latter cases included fibroids, pus-tubes, carcinoma and a few male patients. Jellinghaus and Losee[10] do not deny the defensive ferment theory, but feel that the dialization method is not

6. Williamson: Jour. Obst. and Gyn. Brit. Emp., 1913, xxiv, 211.
7. Oller and Stephan: München. med. Wchnschr., 1914, lxi, Nos. 1 and 2.
8. Schiff, E.: München med. Wchnschr., 1914, lxi, No. 14.
9. Schwarz: Am. Jour. Obst., 1914, lxix, No. 1.
10. Jellinghaus and Losee: Am. Jour Obst., 1914, lxix, No. 4.

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always correct in non-pregnant cases. They therefore do not recommend the test for diagnostic purposes, because errors in technic, responsible for poor diagnosis, cannot be found till after the test. Williams and Pierce[11] conclude that there is an increase of ferment in the blood during pregnancy, but that the ferment is not specific—a similar increase having been noted in other conditions.

Much of the work reported has been done with modifications of the original Abderhalden technic, which renders it totally unfit for comparison either with the original Abderhalden work or with that of other men who used Abderhalden's technic. Many men reported work, without giving detailed account of their technic and control experiments. Such work is of no possible value, and merely serves to increase the confusion that exists regarding the use of the Abderhalden test as a valuable addition to our diagnostic methods in obscure cases.

This work was undertaken to determine:

1. Whether there is a ferment constantly present in the blood of pregnant women that can be demonstrated by means of the Abderhalden dialysis method.

2. Under identical conditions does blood, normal or pathologic, contain a similar ferment which might give the same reaction?

3. If blood, from non-pregnant individuals, gives this reaction, what conditions are most likely to lead to a wrong conclusion when doing the test in a case of suspected pregnancy?

4. Will a quantative estimate of the strength of the reaction assist in differentiating pregnancy from other conditions that might give a positive reaction?

In undertaking this work it was decided to follow Abderhalden's technic.[12] About thirty cases, pregnant and non-pregnant, were tested, in order to become

11. Williams and Pierce: Surg., Gynec. and Obst., 1913, xvi, 411.
12. Described in Beitr. z. Klin. d. Infectionskrankh. u. Immunitätsforsch.

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familiar with the technic, and to be able to avoid the inevitable mistakes of the beginner. These results were not recorded.

In my opinion, at present at least, in order that the results may have a more uniform comparison, all Abderhalden tests should be made according to Abderhalden's technic. Only in this way can a sufficient number of tests be made to permit us to draw warranted conclusions as to the clinical value of the test. Various modifications, at the present state of our knowledge, merely serve to add to the confusion without throwing any real light on the subject.

THE ABDERHALDEN TECHNIC

The technic used in this work is here briefly described:

Preparation of the Placenta.—I was present at the delivery of the placenta and received it immediately after brief examination by the accoucheur. The amnion and cord were hurriedly removed with a scissors, and all large blood-clots washed away. It was then quickly cut into pieces the size of a hazelnut and washed continuously in cold water, with frequent changes and continuous squeezing with the hands till all blood was removed from the pieces. The tissue then looked white, and any pieces containing the faintest tinge of pink were discarded. The washing usually took about two and a half to three hours. The tissue was next transferred to a pan, containing about 1 liter of boiling water, and boiled for five minutes. The tissue was removed, rinsed and pressed repeatedly, and the five-minute boiling repeated. This was continued until 5 c.c. of the filtrate, after boiling the tissue with five times the amount of water, failed to give a reaction with 1 c.c. of ninhydrin on boiling one minute. The tissue had to be boiled and rinsed at least six times; and with some placenta eight and ten boilings were necessary. It was found that some placenta could not be freed from substances that reacted with ninhydrin; and these after repeated boiling had to be thrown away. After the filtrate from the boiling showed absolutely no color on boiling with ninhydrin, the tissue was placed in colored glass jars with chloroform, under toluene. It was reboiled three times and tested with 1 c.c. of a 1 per cent. solution of ninhydrin before each test.

Method of Obtaining Serum.—The blood was taken from the median basilic vein, by means of a clean, dry Luer syringe, and transferred at once to a clean sterile centrifuge

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tube. It was allowed to stand from three to twelve hours till the clot began to separate and press out the serum. It was then centrifuged at about 1,600 revolutions for four to five minutes. The clear serum was pipetted off, care being taken to avoid the formed elements. Hemolized blood was discarded at this juncture to avoid errors in reading the test, due to the ferment set free by hemolysis; 1.5 c.c. of the clear serum was inactivated in the water bath, at 60 C. (140 F.) for one hour and used as a control. In the later experiments 1 c.c. of serum was used according to later advices from Abderhalden.[13] An equal amount of the clear serum was used for the test.

The Diffusion Tubes.—Schleieher and Schull 579 dialyzing tubes were used. These were first tested with albumin to insure their impermeability to it. Any tubes found to let through albumin were discarded. The remaining tubes were tested with a 1 per cent. solution of silk peptone from Hoechst Farbwerke von Meister Lucius and Bruning. These tubes that let through the peptone solution at the same rate, as measured by the depth of color on boiling 10 c.c. of the diffusate with 2/10 c.c. of a 1 per cent. ninhydrin solution, were reserved for future use in making the test. The remaining tubes were discarded. After testing with peptone solution, the tubes were allowed to stand in a liter of distilled water for twenty-four hours in order to remove all peptone solution that might remain in the tube wall. They were then boiled for about two minutes, and stored under toluene in distilled water. They were reboiled for about one minute just before each test.

The Test.—One c.c. of clear serum was introduced into the diffusion tube, together with 1 gm. of placenta protein. Care was taken that all placenta protein was below the surface of the serum to prevent error from decomposition of pieces, adhering to the sides of the dializer, and so not covered by the toluene. The outside of the parchment was carefully washed off with distilled water. The diffusion tube was next placed in a large test-tube, containing 20 c.c. of sterile distilled water. A layer of toluene was placed within and without the dializing membrane to prevent the growth of bacteria and to prevent evaporation.

The Controls.—The controls, with the exception of the first twenty-five to thirty cases, were made exactly the same as the test, excepting that the serum added had been inactivated at 60 C. for one hour. In the first cases the serum alone, without the placenta, and not inactivated, was run as a control. The importance of running an inactivated serum control and placenta is that a negative result obtained thus must mean that there are no dializable substances in the serum, and that the placenta itself is not the source of the

13. Abderhalden: München. med. Wchnschr., 1914, lxi, No. 8.

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dializable substances, and hence must have been properly prepared. The controls were found to be uniformly negative in this series, which, to my mind, is the strongest point in favor of proper technic. In many cases the tubes used as "test-tubes" on one day were used as "control-tubes" on the succeeding day in order to detect leakage of albumin that might occur after a tube had been used several times. Bacteriologic examination was made of the content of the diffusion tubes in cases in which an unexpected positive reaction occurred, in order to rule out the possibility of the digestion products being due to bacterial decomposition. In no case was more than an occasional organism found.

Dialization.—The period of incubation in the first twenty-five to thirty cases was sixteen hours, according to Abderhalden's original suggestion; later it was increased to twenty or twenty-four hours because difficulty was experienced in getting strong enough reactions in the positive cases to admit of a definite conclusion as to whether the test was positive or negative. Abderhalden,[12] later, has advised the same modification of his technic. Ten c.c. of the diffusate from the test- and control-tubes were boiled with 0.2 c.c. of a 1 per cent. solution of ninhydrin. Test and control were boiled over the same flame with the same amount of ninhydrin and as near as possible at the same rate. They were read in all cases after standing one-half hour. All glassware used in the tests was cleaned by allowing to stand over night in potassium dichromate and sulphuric acid, removed, rinsed, and placed in strong potassium hydroxid solution, rinsed in tap-water and then in distilled water, and dried. This was also attended to personally, in order to obviate errors in testing, due to traces of acid or alkali in the tubes.

FINDINGS

Clinical Material Examined.—In all 145 cases were examined.

Pregnancy Cases Examined.—Twenty-nine cases were examined. Most of these were known pregnants at the time of examination and the majority were seven months' pregnant or more. Two cases gave negative reactions. One of these tests, an unknown in the twelfth week after menstration, was made while using the sixteen-hour incubation period; and the error might have been due to the fact that the reaction was so weak that it could not be read. The other was an obscure case in which the uterus was enlarged to the size of a six months' pregnancy but

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no fetal heart-tones could be heard, nor could fetal movements be felt, or fetal outlines be mapped out accurately. The Roentgen ray revealed a shadow of the fetal head above the brim of the pelvis, and the shadowy outline of the vertebral column. The fetus might have been dead, and the reaction negative on that account. I have not had an opportunity to study a case in which the fetus was dead, and do not know how the reaction results under such conditions. The positive reactions varied from very weak to very strong positives. There seemed to be no way of telling which cases would give a weak, and which cases a strong reaction. On the whole, cases from four to six months appeared to give the strongest reactions. Ten cases reacted positively, and six were weakly positive, leaving eleven that reacted strongly positively.

Puerperal Cases.—Twelve cases were examined, varying in time from just after the completion of the third stage of labor, to five months after an abortion. Only one case gave a strong positive reaction, five gave a positive and six a weak or very faint positive reaction.

Lobar Pneumonia.—Nineteen cases of lobar pneumonia were investigated. The reaction was negative in only two cases and in one of these dialization proceeded only sixteen hours and a negative result might be attributed to that. The other was a case in the third day of the disease. On the whole, the five cases examined before the crisis gave considerably weaker reactions than those examined just after the crisis.

Acute Rheumatic Fever.—Having in mind the close relationship of the causative organisms of pneumonia and rheumatism, it was decided to test out the proteolytic power of the serum in the latter disease, as well as in the former. Eight cases in all, including acute and convalescing cases, were examined. The reaction proved negative in one out of the eight tests. One test gave a strong positive. The other six gave a weak or very weak positive test.

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Typhoid Fever.—Five cases were examined, varying from the second to the sixth week. The reactions varied from a faint trace to a weak positive in all cases, and were apparently more marked later in the course of the disease.

Tuberculosis.—This was next investigated. Six cases were examined, all in advanced stages of pulmonary tuberculosis, according to the clinical diagnosis. One of these gave a negative result, the other five a weak positive or a positive reaction.

Nephritis.—Eleven cases of nephritis were examined. They were mostly of the chronic interstitial type. Nine gave a positive reaction, two, weakly positive and one a negative reaction. Two cases at the verge of uremic coma gave strong positive reactions.

Carcinoma.—Eight cases were examined: six carcinoma of the uterus, one carcinoma of the stomach and one carcinoma of the bladder. Two negative reactions were obtained, two were positive and four weakly positive. There seems to be little, if any, relation between the extent of the carcinomatous involvement and the strength of the reaction.

Meningitis.—Five cases of meningitis were examined. These included two epidemic, one syphilitic and two tuberculous forms. The reaction was negative in the syphilitic, one of the tuberculous and one of the epidemic forms. The other tuberculous and epidemic cases gave a faintly positive reaction.

Diabetes.—Five cases of diabetes mellitus were examined, in various stages of the disease. One gave a negative reaction, two a faint positive, one strong positive and one medium positive.

Fibroids.—Four cases were examined. Two were negative, one gave a very faint reaction and one a positive reaction. In the latter case sufficient time had not elapsed since the test absolutely to rule out the possibility of a pregnancy being coexistent.

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Blood and Nervous Diseases.—Under this head are considered two cases of pernicous anemia, which gave weak positive reactions; one splenomyelogenous leukemia, which gave a strong positive reaction; one malarial infection, a few hours after the chill, which gave a strong positive test. One case of hypopituitarism gave a faint trace. One case of head tetanus, in the seventh day of the disease, gave a weak positive. One dementia praecox gave a trace. One case of scurvy in the convalescent stage gave a negative reaction. Three cases of syphilis were examined, all in the latent stages; two were found positive and one strongly positive. One alcoholic neuritis was strongly positive.

Normal Cases.—Seventeen normal or patients suffering from minor affection, not giving systemic reactions, were examined. Thirteen gave negative tests and four were positive. Of the latter, one had had a light meal one hour before the test, one was a case of chronic constipation, two were hernia cases just before operation, and it is possible that these patients might have eaten more than they admitted.

One case of suspected chorio-epithelioma was examined. The woman had passed a hydatid mole one year previous. She had flowed on and off ever since. The Abderhalden test was positive, but scrapings from the uterus failed to show malignant changes or evidence of recent pregnancy.

SUMMARY

From the results it would seem that there is a ferment present in the blood of pregnant and puerperal women in the vast majority of cases, and that it can be demonstrated by the Abderhalden dialysis method.

Under the same conditions normal blood sometimes, and blood from various pathologic conditions frequently, gave the same reaction. The strength of the reaction varied, with some exceptions, with the disease present in the case examined.

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A large number of pathologic conditions may give a positive reaction; and hence would have to be ruled out when applying the test to a given individual for the diagnosis of pregnancy.

Quantitative estimation of the strength of the reaction is of little value in differentiating pregnancy from other conditions that give a positive reaction.

Abderhalden[14] states his position very definitely on the reaction in non-pregnant individuals. He says:

> The serums from Gravid and non-gravid individuals differ from each other, among other things, in that the serum of the former contains a ferment that will digest placenta protein. This statement holds at least for normal non-gravidae. According to my experience and that of many other investigators this statement holds for pathologic non-pregnant cases also.

I have been unable, however, to find any data by him of a systematic investigation of other pathologic conditions using the same technic as used in the pregnancy test.

From these results it appears to me that the best working hypothesis to explain the phenomena noted here is the following:

In many conditions of altered metabolism, whether due to infection, malignant disease, pregnancy, the involution period of the puerperium, kidney lesions or certain nervous diseases, there is an abnormal amount of ferment in the blood. This may be due to:

A. Absorption of ferment by the blood from some source in the body, where a great amount of ferment activity is going on. Six such sources may be recognized by this work:

1. In pneumonia, especially after the crisis, when the lobes of the lung are undergoing resolution.

2. In pregnancy when there is a constant transfer of nutritive material from the mother to the fetus, and of waste material from the fetus to the mother,

14. Abderhalden and Fodor: München. med. Wchnschr., 1914, lxi, No. 14.

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and the whole process carried on by anabolic and catabolic ferments in the chorionic cells. It is easy to conceive how absorption could occur from such a fetal structure so rich in ferment as the placenta.

3. In carcinoma, in which apparently abnormal ferment activity is going on in a poorly defined mass of tissue, which is invading and breaking down other tissue.

4. At the height of digestion, at which time there is absorption of the products of digestion and a large amount of fluid containing an immense amount of ferment is bathing the surface of a rapidly absorbing membrane. Were the positive Abderhalden tests when the serum is obtained at the height of digestion due to amino-acid content of the blood at the height of digestion, I fail to see why the inactivated serum would not contain just as many dialyzable substances as the active serum and therefore the control give the same color reaction as the test.

5. In malaria, when there is, at the time of the chill, a great destruction of red cells with the setting free of ferment.

6. In splenomyelogenous leukemia, in which there is a great increase in the formation of and destruction of white cells and destruction of the red cells.

B. In the acute infections recovery is probably due to the mobilization of the ferments by the body-cells, which either prevent the development of the bacteria or actually digest them and their toxins. Tuberculosis being in the advanced stages, almost always associated with secondary infection, might be considered as coming under the same heading as acute infectious diseases.

The reason for the reaction in diabetes is less apparent, but with the altered functions of the pancreas one might expect to find various disturbances in the tryptic or antitryptic power of the blood.

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The positive reaction in chronic nephritis is not so easy to explain by this theory, but it is known that there is profound alteration in protein metabolism, which is essentially carried on by proteolytic ferments; and therefore it would not be surprising to find an abnormal amount of these in the blood-stream.

These points are merely mentioned here, and are not to be regarded as definite conclusions. It is my wish to go deeper into the subject, to see a wider range of diseases, and to examine more cases from each before trying to formulate definite conclusions. It is hoped that other workers will investigate along similar lines; and that such investigations will enrich out knowledge of the underlying principles of physiology and pathology.

I wish to express my thanks to the resident and attending staffs of the Cook County Hospital for access to the clinical material, without which this work would have been impossible. Also to Drs. D. J. Davis and C. S. Bacon for timely suggestions and helps throughout the work.

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ABSTRACT OF DISCUSSION

ON PAPERS OF DRS. BALL AND FALLS

Dr. Charles Goodman, New York: I have transplanted kidneys and thyroids in dogs, and while the autotransplants were successful, my homotransplantations were followed by autolysis; this occurred although I was able by special technic to overcome the tendency to physical injury observed in the transplant during the stage of vasomotor paralysis. I have used the Abderhalden method in determining the specific ferments in the blood. I found, in a series of fourteen cases of transplantation of the thyroid gland, that, in nine instances, a ferment could be isolated by the dialysis test which was capable of digesting suprarenal tissue. I intend to determine by the Abderhalden method what other organs are susceptible to vital insult following homotransplantation.

Dr. M. G. Wohl, Philadelphia: I have applied the Abderhalden test in cases of pregnancy and carcinoma but chiefly in nervous diseases. My material was obtained from the Philadelphia General Hospital, the epileptic colony of Spring City, and from wards of the Samaritan Hospital. The tech-

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nic was followed closely in all details as laid down by Abderhalden. In twenty-two cases of pregnancy I found the test positive. The patients varied in term from three to nine months. Cases of toxemia of pregnancy gave surprising results. In one case of eclampsia, placenta gave a faint rosy color and liver tissue strongly positive. Three patients with pernicious vomiting gave the same faint reaction with placenta, and moderate violet with liver. I applied this reaction in cases of dementia praecox and epilepsy at various times, between the attacks, etc., and in some cases I have been fortunate enough to obtain the blood five minutes after the attack, and I find that if the technic is correct in all details, we get uniform results. In cases of dementia, and epilepsy which advanced to dementia, I have obtained a positive reaction with brain and testicular tissues if the serums were taken from males; and with brain and ovarian tissues with serums from female patients. Dr. Falls obtained a positive reaction with placenta in some cases of dementia. I recall a similar case of positive reaction with placenta in an epileptic male, 17 years old. When I obtained this reaction, I went over my technic again and was convinced that there was no error in it. After careful investigation I found that a day and a half before the blood was taken the patient had suffered an injury of his arm. This gave me the clue to the situation. As you know, through hemolysis of blood-corpuscles, a ferment is liberated which is non-specific and this non-specific ferment was very likely responsible for the cleavage of the placenta. The reason for obtaining negative reactions from inactivated serums taken soon after a meal, which according to Dr. Falls should be positive, is the fact that the amino-acids present in the blood after a meal are easily diffusible and are found in the circulation in such small quantities that they would hardly give a positive reaction with the ninhydrin.

Dr. C. P. McCord, Detroit: I began working with this test in the fall of 1912 and since that time have performed nearly two thousand tests. These have been in part clinical and in part laboratory tests. They have been distributed over pregnancy, tuberculosis, goiter, cancer, a few cases of syphilis and laboratory experiments with organ-tissues to determine the specificity of organ-proteins. This work when performed with proper regard for the intricacies of the technic has yielded results corresponding to the clinical condition in a high percentage of cases. Proper regard for the technic presupposes careful testing of the parchment thimbles so that they are of known accuracy. These should never be actually boiled but merely brought up to the boiling point. Further, these thimbles should be allowed to cool before using, for if serum is placed in heat-expanded thimbles, the serum passes into the spaces in the parchment and, when

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the thimble cools off and contracts, the serum may be forced to the outside of the thimble and thus vitiate the tests. I have used this test in a few cases of carcinoma of the thyroid in dogs. I find that serum from dogs with cancer of the thyroid reacts not only with carcinomatous thyroid gland but with normal thyroid tissue as well. This is to be expected, for carcinoma retains some of the characteristics of the cells from which it develops. The intensity of the color reaction with normal thyroid tissue was much less than with carcinomatous thyroid. Moreover, when the serum from these animals was tested with tissue from other organs, for instance, kidney or ovary, positive tests were obtained. Although the serum alone is in many instances dialyzable, the serum plus any organ tissue yields a positive reaction of greater intensity than the serum alone, but of less intensity than the serum plus cancer thyroid tissue. This, I consider very plausible. While I believe that the parenchymatous proteins of an organ are specific, I also believe that the connective-tissue stroma of the organs is non-specific. In destructive lesions of organs such as the thyroid cancer, the stroma may be altered as well as the parenchyma and non-specific as well as specific ferments arise. These are capable of digesting the non-specific parts of other organs. I believe that herein is a method that is very useful within certain limitations. The method is not infallible. After the technic was carefully controlled, 5 per cent. of my cases gave erroneous results. There are certain natural limitations. For instance, in 140 cases of tuberculosis (these I have carried out collaborating with Dr. Lockwood), 25 cases in the cavity stage were uniformly negative. Apparently the serum in this stage contains no protective enzymes. Withal, I think this a most useful method in diagnosis and likely to prove helpful in many ways.

Dr. C. F. Ball, Rutland, Vt.: The technic described by Dr. Falls is practically mine. My own, however, has been the half method of Schwartz instead of the 10 c.c. of Abderhalden. I have guarded that by using 0.2 c.c. of ninhydrin to 5 c.c. of my solution instead of the usual quantity of ninhydrin, which would give me an error if I were to get one, more readily than using 0.1 c.c. All of my tissue has been properly prepared, as has been suggested, and when I can't get it clean by half a dozen boilings, using 1 c.c. of the ninhydrin to 5 c.c. of the boiling water, I throw it away as useless. The time I have taken in preparing my tissue, as has been stated, is anywhere from one to two hours, depending on the tissue. I have prepared tissue and have boiled it a dozen times and couldn't get it satisfactory. I have also boiled tissue a dozen times and got it apparently satisfactory and when used it would give treacherous reactions and I would have to throw it away. I never have used tissue

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until I have run it as a control with a known condition and tried out fundaments at least two or three times before I depended on it for new work. At first I used water distilled at the time of making the test, but in the latter part of my work I have used water distilled at or near the time of making the test and then boiled. Normal tissue of the gland invaded would be expected to give a reaction along with the malignant tissue used, as Dr. McCord has found. In a sarcoma of the thyroid normal thyroid tissue used also showed the reaction. The quantitative determination, spoken of by Dr. Carlson, is to be hoped for. When we get the quantitative determination, we will then find that there is a difference in the amount of ferment produced by the apparently healthy or normal person afflicted with a malignant infection, as compared with the patient who has passed on to the latter stages of the disease in which the ferment is largely reduced, the system having given up the fight, which accounts, I think, in a few reported cases, for the lack of a positive reaction in grave malignant cases just prior to death.

Prof. A. J. Carlson, Chicago: The explanation offered by Dr. Falls of the positive reactions in non-pregnant cases is probably the correct one. I do not believe that the position taken by Abderhalden, namely, that normal blood is free from polyvalent proteolytic enzymes, is tenable. The Abderhalden test must therefore be a quantative one, and unless the test can be made a quantitative one, it will be of limited value. I dare say the qualitative test would be positive in 100 per cent. of normal cases if the time of digestion is extended. We have polyvalent ferments in the blood of normal animals and any condition which involves a rapid impairment of the tissues leads generally to liberation of an increased amount of ferments into the blood. Hence, a greater digestion in certain diseases. Abderhalden has done a very great service for biology and medicine by this extension of the anaphylaxis and immunity phenomena, but I think some workers have been led astray by his view that normal blood is free from non-specific or polyvalent enzymes. Furthermore, the Abderhalden test depends, of course, on a reaction to proteins similar to that to antigens in immunity, and the intensity of the immunitary reaction varies greatly in different individuals.

Dr. F. H. Falls, Chicago: I think Dr. Ball is wrong. Abderhalden uses 5 c.c. instead of 10—that is the technic I have been following—5 c.c. of the filtrate of the boiled water plus placenta. In the few toxemic cases that I have examined, I found, as Dr. Wohl did, a weakness of the reaction. One patient with puerperal insanity which developed about four or five days after the delivery was examined. She gave a very weak positive reaction. That

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might have been due to the fact that in the puerperal state the reaction is usually weakened. I don't know. I have had no chance to examine any epileptic cases. Did Dr. Wohl use placenta as substrate in his epileptic cases? I think there is some slight misunderstanding about my statement of the amino-acid contained in the blood giving a positive Abderhalden reaction. My position is this: If the blood is taken at the height of digestion in an Abderhalden test, and 1 c.c. of inactivated serum and 1 c.c. of active serum are put in separate dialyzation thimbles, with the same amount of protein in each thimble, then if you get a positive reaction in your test and a negative reaction in your inactivated serum control, it cannot mean that the reaction is due to amino-acid in the serum. There must be ferment activity in the active serum test, and no ferment activity in the inactivated serum control. The criticism that I have heard, heretofore, has been that boiling renders the thimbles thicker and less permeable. Concerning Dr. McCord's point about cooling the thimbles, after boiling the protein was put into the thimble first and then the serum. The thimble had cooled to very nearly room temperature when the serum was introduced. My controls, which were uniformly negative, had the same amount of serum, and were handled in exactly the same way.

Dr Carlson's idea of the quantitative estimation of the reaction is a very good one. Dr. Welcher and I, at the University of Illinois, tried to split up the placenta by various chemical methods in an endeavor to find a pure placenta protein which would react more delicately with the serum from pregnant women. We hoped to find a protein in the placenta for which the ferment would be specific. We were unable to do so. I believe that Dr. Ball's modification of using 5 c.c. of the diffusate in testing is wrong. This is a quantitative reaction, absolutely. I believe that we must all use the typical Abderhalden technic without modification and put down our results as we find them. That will soon tell us about the reliability of the test not with ten or a dozen cases, but with hundreds and thousands of cases. Then we will get results that will be reliable, and comparable. I do not wish to be misunderstood concerning the value of the test. I think that in any obscure case it would be valuable when there is a question of pregnancy. The point is this: If we can get a test that will be reliable in even 80 per cent. of cases, we have no right to disregard such a test, and say it is of no value. The Wassermann and other tests are far from infallible, but nevertheless valuable.

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ADENOMYOMA OF THE RECTOVAGINAL SEPTUM*

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NEW YORK

Prior to the publication, in February, 1913, of reports of Cuthbert Lockyer's cases of adenomyoma of the rectovaginal septum,[1] the occurrence of such growths in this region had apparently not been observed either by surgeons or by pathologists.

During the month of January, 1913, during which month Lockyer reported his cases, there were sent to me for diagnosis two rectovaginal tumors in which complete hysterectomy had been done. These proved to be counterparts of Lockyer's cases. It is through the courtesy of Dr. William S. Bainbridge, who operated on the patients, that I have the opportunity of reporting the cases.

CASE 1.—Mrs. D., aged 36, was married but had had no children. There was no history of syphilis. One year previously she had been operated on for extra-uterine pregnancy. One small fibroid and the appendix were removed at this time. Menstruation had been regular up to three years ago, when she began to have dysmenorrhea and irregular flow. The present illness began two years ago with a gradual onset. The chief complaint was excessive menstruation, vaginal discharge and pain in the lower part of the abdomen. There had been loss of weight. Examination on admission to the hospital showed a mass between the uterus and rectum and attached to each. The uterus was enlarged and attached to the bladder and rectum. Cervix was ulcerated. The diagnosis was made of a "precancerous" uterus with probable malignant mass in culdesac.

Operation was performed, Jan. 9, 1913. Many pelvic adhesions were found. Complete hysterectomy was done

* From the Research Department of the New York Skin and Cancer Hospital.

1. Lockyer, Cuthbert: Proc. Roy. Soc. Med., 1913, vi, No. 4.

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with removal of pelvic lymph-nodes and ligation of the right internal iliac artery. In the culdesac a bridge of carcinoma-like tissue was found extending from the vesicovaginal junction posteriorly to the rectum and so blended with the latter that a partial excision of the wall of the rectum was necessary. The patient made an uneventful recovery, going home in four weeks and has been well ever since.

Pathologic Report 2018: The tissues consist of the uterus, tubes and ovaries, portion of the rectum and pelvic nodes. The uterus is 8 cm. long and 6 by 5 cm. at fundus. The ovaries are enlarged and cystic, the largest 5 cm. in diameter. The uterine wall shows a fibroid 2 cm. in diameter. To the lower portion of the posterior wall of the uterus there is attached by a band of fibrous tissue a section of rectal mucous membrane measuring 3 by 2 cm. (Fig. 1).

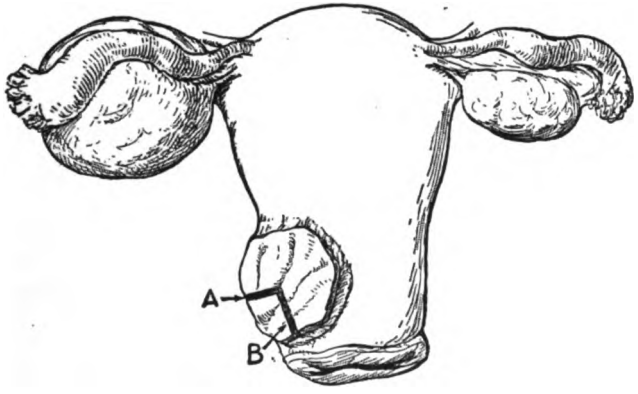

Fig. 1.—Adenomyoma of the rectovaginal septum, Case 1. A diagrammatic representation of the relation of the rectal tumor to the posterior uterine wall. Horizontal section taken at *A* is shown in Figures 2 and 3.

The rectal mucosa appears normal, but the tissue between it and the cervix is dense, and cuts like a fibroid.

Microscopic examination of the sections of the uterine body and cervix fails to show any malignant new growth. There is a chronic cervicitis and the pelvic nodes show chronic inflammatory changes. A horizontal section through the rectal tumor (Fig. 2) shows normal rectal glands and stroma and beneath this a broad zone of non-striated muscle. Scattered through the muscle are glands occurring singly or in groups, some lying within 1 mm. of the rectal mucosa. They are surrounded by a stroma of small round and oval cells. This stroma is more in evidence where there are several glands together. Some of the glands are enlarged or cystic and contain blood-cells and exfoliated epithelium, the result of hemorrhages into them. The epithelial cells lining the

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glands are cylindrical, and cilia can be demonstrated on the surface of some. The glands resemble those of the uterine mucosa rather than those of the rectum.

CASE 2.—Mrs. F., aged 40, married, has two children, aged 17 and 13. She has never been seriously ill. The movements of the bowels have been irregular until three months ago when they became regular. She does not know what brought about the change. Ever since marriage there has been pain on the left side low down in the region of the ovary. This pain comes and goes without relation to the menstrual

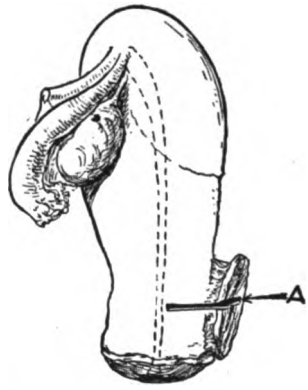

Fig. 4.—Adenomyoma of the rectovaginal septum, Case 2. A diagrammatic representation of the relation of the rectal tumor to the posterior wall of cervix. Horizontal sections taken at *A* are shown in Figures 5, 6 and 7.

periods. Hemorrhoids have given her trouble for the past thirteen years and recently there has been bleeding from the rectum. Physical examination showed external and internal hemorrhoids, tenderness over the appendix and a large uterus with a lacerated cervix. A distinct hard mass was felt in the culdesac of Douglas, beginning to adhere to rectum. It was thought to be a uterine fibroid. Examination of this mass from the rectal side with a proctoscope showed an intact rectal mucosa but one which bled readily at the site of the tumor.

Jan. 21, 1913, hysterectomy was performed with removal of the tubes and ovaries and appendix and a portion of the rectum. It was found that between the uterus and the rectum attaching together their peritoneum and muscular coats was a nodule ¾ inch in diameter with contracted tissue around it strongly suggesting malignancy. It was necessary to excise a piece of rectum to remove the growth.

Pathologic Report 2042: The uterus is 9 cm. long and 5 by 4 cm. at fundus. The wall is 2 cm. thick and the mucosa

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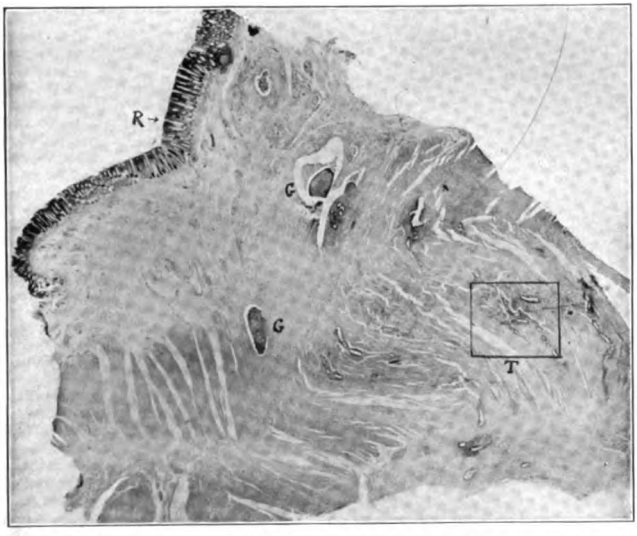

Fig. 2.—Adenomyoma of the rectovaginal septum, Case 1. Low-power view taken at *A*, Figure 1. At *R* is seen normal rectal mucosa. At *G* is an enlarged gland showing hemorrhage and exfoliation of epithelium into lumen. At *T* is a blocked-out area of glands and stroma shown in Figure 3.

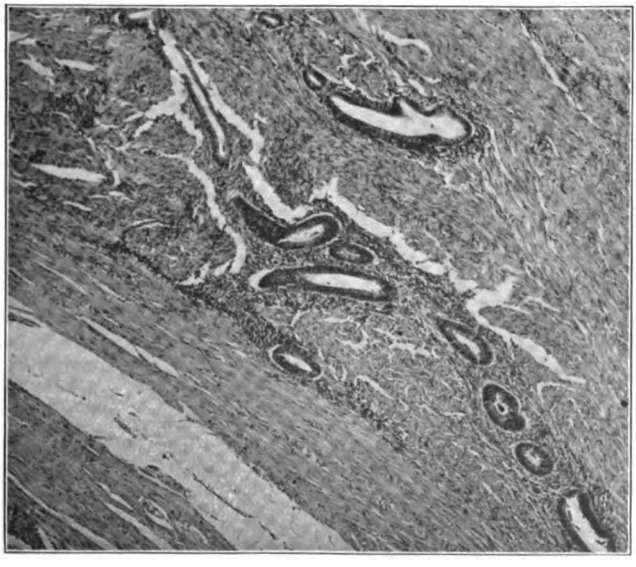

Fig. 3.—Adenomyoma of the rectovaginal septum, Case 1. High-power view of blocked-out area *T*, Figure 2. This shows islands of mucosa with the glands and stroma characteristic of uterine mucosa.

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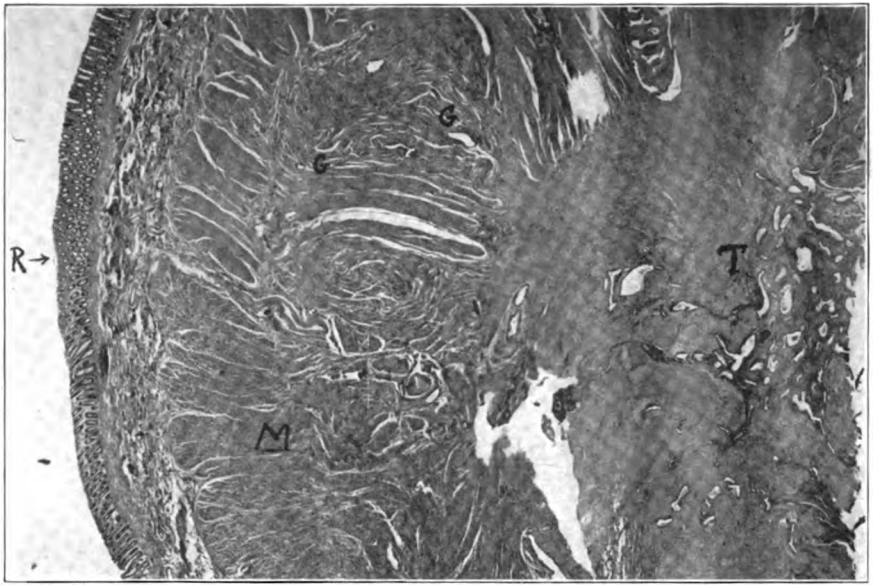

Fig. 5.—Adenomyoma of the rectovaginal septum, Case 2. Low-power photograph showing normal rectal mucosa at letter *R*. Beneath this is a growth of smooth muscle at *M*, with a few scattered glands at *G*, and a large group of them at *T*.

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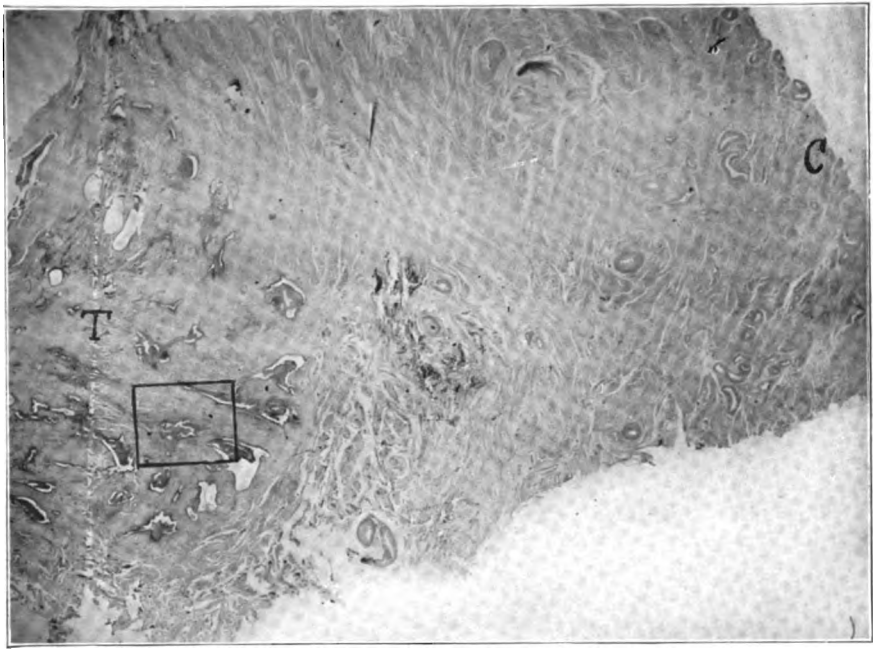

Fig. 6.—Adenomyoma of the rectovaginal septum, Case 2. Low-power view continuation of mass of glands and stroma seen at letter *T*, in Figure 5, and indicated here also by letter *T*. Blocked-out rectangular area is shown in the high-power view of Figure 7. Letter *C* is at the uterine edge of section at a point 0.5 cm. from the cervical canal.

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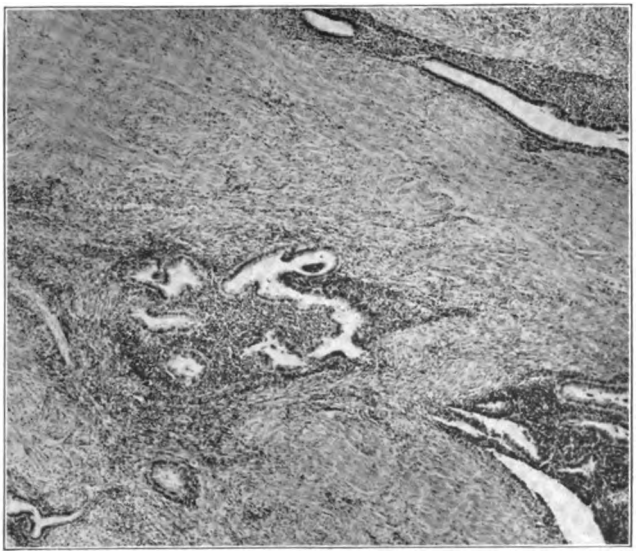

Fig. 7.—Adenomyoma of the rectovaginal septum, Case 2. High-power photograph of blocked-out area seen in Figure 6. Compare with the high-power view of the glands and stroma of Case 1, shown in Figure 3.

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roughened. Both ovaries are cystic and enlarged. Tubes are thickened at fimbriated end and adherent to ovaries. There is a small area of rectal wall attached posteriorly to uterus by a band of fibrous tissue (Fig. 4). On section there is a hard fibrous base from 0.5 to 1 cm. deep lying beneath rectal mucosa.

Microscopic examination of sections of the uterus shows a chronic cervicitis. There are moderate inflammatory changes in the tubes and appendix. Rectal mass: sections were cut horizontally through from the rectum to within 0.5 cm. of the canal of the cervix (Figs. 5 and 6). Here as in Case 1, the rectal mucosa is normal. Beneath it is a growth of smooth muscle which extends through and is continuous with that of the cervix. Glands appear within 2 mm. of the rectal epithelium. They occur singly at first with very little stroma, but further in there is grouping of large numbers of them and at a depth of 1 cm. they form a nodule from 1 to 1.5 cm. in size. The epithelial lining cells are cylindrical and of the uterine type and cilia can be demonstrated. Between this group of glands and the cervical mucosa there is a distance of 2 cm. in which no glands can be seen. There is not so much cystic dilatation of the glands as in Case 1, but some show blood and detached epithelium as evidence of hemorrhage (Fig. 7).

DIAGNOSIS

These two cases presenting the same picture clinically and pathologically can be considered together from the point of view of diagnosis. While the microscopic picture was identical with that of cases of adenomyoma of the uterus which we had previously seen, the unusual location and the absence, at that time, of any records of such cases in the literature, led us to ask the opinion of Dr. Thomas S. Cullen, who on account of his well-known book[2] and other publications on the subject of adenomyomas, would be in a position to confirm our diagnosis if correct. Dr. Cullen very kindly examined the slides that I mailed him and wrote me very fully his opinion of the cases. Since then he has published reports of two cases of his own with a review and discussion of Lockyer's cases.[3] I cannot do better than quote a portion of Dr. Cullen's views with regard to our cases. He writes:

2. Cullen, Thomas S.: Adenomyoma of the Uterus, Philadelphia, W. B. Saunders Company, 1908.
3. Cullen, Thomas S.: Adenomyoma of the Rectovaginal Septum, The Journal A. M. A., March 14, 1914, p. 835.

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There is not a shadow of a doubt but that we are dealing with adenomyoma of the uterus in both cases. From what I can gather the growth is undoubtedly of the uterine type and not of the rectal and the fact that the rectal mucosa is perfectly normal and in no way encroached on also favors the idea of the uterine origin. Furthermore, if we are dealing with an adenomyoma of the rectum then we should expect the glands scattered throughout the tumor to consist of rectal glands and not uterine. Both of these growths are essentially adenomyomas of the uterus and they have undoubtedly involved the rectum secondarily. As you know, a certain number of these adenomyomas are particularly prone to form adhesions. This would naturally be more frequent in the cervical portion because the uterus there lies in close contact with the rectum.

From the point of view of clinical diagnosis these tumors are of interest. Dr. Cullen has shown that some adenomyomas can be diagnosed by their increase in size at the menstrual period with accompanying pain and discomfort. This is doubtless due to congestion and hemorrhage such as is shown in our cases in which the glands contain blood-cells and epithelium. Examination of the histories, however, does not show that discomfort or pain if present was marked enough to be noted by the patients.

These tumors, while benign, have on physical examination the characteristic feeling of malignant growths on account of their density and tendency to form adhesions, and in both of our cases other surgeons had previously examined the patients and pronounced their condition inoperable on account of the apparent involvement of the rectum by a malignant growth.

That they may occur should be recognized by the surgeon, as the diagnosis can be made microscopically by a preliminary section. A simple excision of the mass would then cure the patient and there would be avoided the more serious operation of hysterectomy which would have been indicated if clinical diagnosis alone were relied on.

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CANCER OF THE PROSTATE*

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The facts that the general subject of cancer presents even to-day one of our most serious and unsolved problems; that cancer of the prostate is a comparatively frequent and exceedingly distressful form of the disease; and the probability that by a more widespread application of the present available knowledge of this pathologic process the prevalent high percentage of disability and death would be considerably diminished, are advanced as sufficient reason for presenting a discussion of the subject with a contribution of data to the fund of experience.

The pathogenesis of cancer of the prostate is still a debatable question; the frequency of its occurrence, the usual sites of its original focus, its mode of extension and metastasis, are phases of the subject which are reasonably supported by facts.

This paper is based, first, on the examination of a series of 716 prostatic specimens removed at operation in the Mayo Clinic from Oct. 28, 1904, to June 1, 1914; secondly, on the pathogenesis of epithelial tumors of the breast, as view by MacCarty; and thirdly, on a review of the literature.

PATHOGENESIS

The opinions of various investigators with regard to the pathogenesis of cancer of the prostate are not uniform. The cause of this lack of uniformity seems to be due, at least in a great measure, to the different points of view from which the pathologic process is viewed.

* From the Mayo Clinic.

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Some authors maintain that cancer does not begin in a hypertrophic portion of the gland, that its presence here is an invasion from a focus situated elsewhere in the organ, usually in the posterior lobe. Others state that cancer occurs as an almost imperceptible transition from a benign adenoma. Still others, disregarding as unessential the consideration whether the associated process be one of general hypertrophy, adenoma or other pathologic condition, view, apart from its causation, the pathogenesis of prostatic cancer, likewise of cancer in general, as fundamentally a question of the histogenesis of epithelial cells.

A summation of the evidence adduced from the various investigations emphasizes the fact that cancer occurs in the atrophic, the apparently normal and the hypertrophic prostate. In the light of this accepted fact is seen an indication that involved in the development of the cancerous process there is a study far more basic than what is comprehended in a consideration of associated pathologic conditions. Again, the weight of evidence seems to prove that the disease begins most frequently in the posterior lobe of the gland. This seeming selectiveness of the process, however, is no more explicable in the prostate than are similar phenomena in other organs. The incident of original focus is aside from the underlying principle of pathogenesis, as advanced in the theory of the histogenesis of epithelial tissue.

CANCER OF THE BREAST

In 1898 Albarran and Hallé,[1] in an oft quoted and adversely criticized treatise under the caption, "Hypertrophy and Epithelial Neoplasms of the Prostate," direct attention to the "remarkable *analogy between the hypertrophied prostatic gland and the mammary gland* affected with benign tumors, or with those epi-

1. Albarran and Hallé: Ann. des mal. des org. génito-urin., 1898, p. 797.

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thelial and interstitial lesions, which are still wrongly classified among the benign neoplasms and the chronic inflammations."

In 1907, Ewing,[2] discussing "Cancer Problems," stated that "inflammatory hyperplasia passes by insensible gradations into neoplastic growths."

During the past five years, MacCarty[3] has contributed several papers to the subject of cancer of the breast. This author was stimulated to undertake an extensive investigation of the epithelial growths of the mammary gland because of inability to harmonize his daily observations with the prevalent views of the pathogenesis of these tumors and the apparently redundant, diverse and unintelligible classification of the pathologic processes.

At the beginning of this work the hypothesis was formulated, that the cells of cancer are the direct offspring of cells normally present in the acinus of the breast, and that they are derived neither from the differentiated cells through metaplasia, nor from prenatal or postnatal rests, according to the views attributed to Cohnheim and Ribbert. The investigation has been pursued on the foundation constructed by authoritative embryologists; personal speculation has been scrupulously avoided and notes of conditions have been confined strictly to the morphologic evidence presented by epithelial tissue under the microscope.

At the present period of this long investigation, MacCarty states that his earlier observations have been confirmed, and that his view of the origin of cancerous cells in the breast is no longer a hypothesis, but a demonstrable fact.

With the purpose of endeavoring to determine an analogy between the pathogenesis of cancer of the mammary gland, as advanced by MacCarty, and the

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2. Ewing: Harvey Lectures, Nov. 16, 1907.

3. MacCarty: Tr. of Surg. and Gynec. Assn., 1910; Surg., Gynec. and Obst., October, 1913, p. 441, Surg., Gynec. and Obst., March, 1914, p. 284.

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pathogenesis of cancer of the prostate, I undertook the examination of this series of prostatic specimens.

A brief summary of MacCarty's investigation of epithelial growths of the breast follows:

The normal acinus of the mammary gland is lined with a single row of cylindrical epithelial cells, which are the differentiated, functional cells of the organ. These differentiated cells arise from the stratum germinativum, which is usually undiscernible until some stimulus causing hyperplasia is present. Then the germinative layer appears in the acinus as a second row of cells which adjoins immediately the surrounding stroma. For descriptive purposes these cells are termed the outer row and are described as the undifferentiated or partially differentiated precursors of the specific functional cells. The morphologic appearance of these cells of the outer row differs from that of the differentiated cells, which now form and are termed the inner row. In the presence of an excessive or abnormal stimulus the cells of the outer row become hyperplastic and partially or completely fill the acinus. In this event, the functional cells are present, exfoliated or absent. Furthermore, the undifferentiated cells are observed not only intra-acinic, but also extra-acinic, situated in the stroma. Therefore, some microscopic fields present both rows of cells, differentiated and undifferentiated, distinct in the acinus; other fields show a definite predominance of intra-acinic, hyperplastic undifferentiated cells; and in still others, both intra-acinic and extra-acinic hyperplastic undifferentiated cells are seen. In some fields in which the inner row of functional cells are exfoliated or absent, and the undifferentiated cells of the outer row hyperplastic, the line of demarcation between the acinus and the stroma is observed obliterated, totally or in part, and the latter cells are noted also in the stroma. The facts that epithelial invasion of stroma is the accepted histologic picture of cancer; that in these observations

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the epithelial cells in the stroma are morphologically indistinguishable from the hyperplastic undifferentiated cells of the acinic outer row; and finally, that the latter cells represent the normal precursors of the functional cells in the stratum germinativum, seem to lead logically to the deduction that *the cancerous cells of the mammary gland are the direct, immediate descendants of the undifferentiated cells of the germinative layer,* consequently, that the hypothesis of "embryonic rests" is not essential for the explanation of cancer in this organ.

For the sake of simplicity in studying the mammary epithelial tumors, the following classification is based on the observations cited above: The pathologic processes involved in these epithelial growths are designated, (1) primary hyperplasia; (2) secondary hyperplasia; (3) tertiary or migratory hyperplasia. In primary hyperplasia, both rows of cells, the inner, differentiated, and the outer, undifferentiated, are present and concerned in the reaction; in secondary hyperplasia, the outer row, undifferentiated cells, are excessively proliferated (cancer?); in tertiary hyperplasia, the hyperplastic undifferentiated cells of the outer row are also present in the stroma (cancer).

The probability is advanced that, viewed in the light of these studies, the old criterion for determining cancer histologically, namely, the permeation of the "basement membrane," may be erroneous, consequently, that we may be compelled to view the condition of secondary hyperplasia as possibly malignant before invasion of tissue has occurred.

CANCER OF THE PROSTATE

In examining the prostatic specimens of this series for the purpose of seeking an analogy between the cancerous process in this gland and that noted by MacCarty in the breast, the following observations have been made:

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The normal acinus of the prostate is lined with a single or an imperfect double layer of columnar epithelial cells. These are the differentiated or specific functional cells of the gland. In the examination of the pathologic specimens, these functional cells are seen frequently in a state of excessive proliferation, forming projections in and bridges across the acini. There is observed also partial or complete exfoliation of the same cells with the formation of cysts, which are empty or contain accumulations of the cells in varying degrees of degeneration. Some fields are noted in which the acini present not only the proliferated functional cells, but also, outside these and immediately adjoining the stroma, another row of cells, which are morphologically dissimilar to the inner row of differentiated cells. Other fields contain acini with functional cells present, exfoliated or absent, and the lumina partially or completely filled with the hyperplastic undifferentiated cells of the outer row. Still other fields are seen in which these hyperplastic cells of the outer row are both intra-acinic and extra-acinic, consequently presenting epithelial invasion of tissue, the accepted picture of cancer. Furthermore, what is impressive, the three conditions just described have been observed together in the same microscopic field.

An analysis of this comparative study of the prostate and the mammary gland indicates that an analogy exists between the microscopic pictures of epithelial changes in the cancerous process of both organs. The analogy being accepted, it follows that this study of the prostate adds further support to MacCarty's deduction, drawn from extensive observations on epithelial growths of the breast. If adequately prolonged and comprehensive investigations in the future positively confirm the present view of the latter author, it may be stated of the prostate that, whatsoever the associated pathologic condition or wheresoever the original focus, these are factors which are unessential

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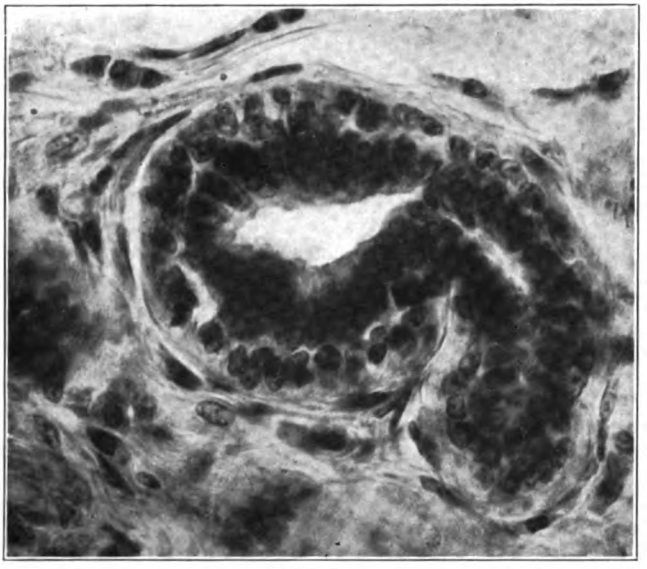

Fig. 1.—Breast; acinus showing primary epithelial hyperplasia (MacCarty).

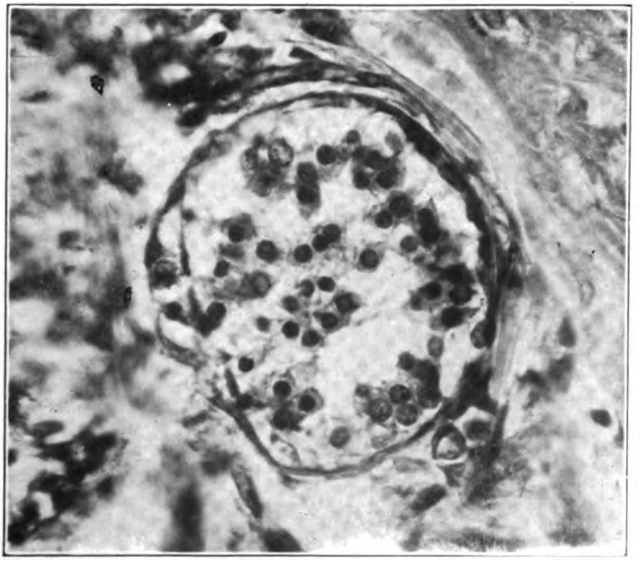

Fig. 2.—Breast; acinus showing secondary epithelial hyperplasia (absence of inner row of cells and hyperplasia of outer row of cells in lumen [MacCarty]).

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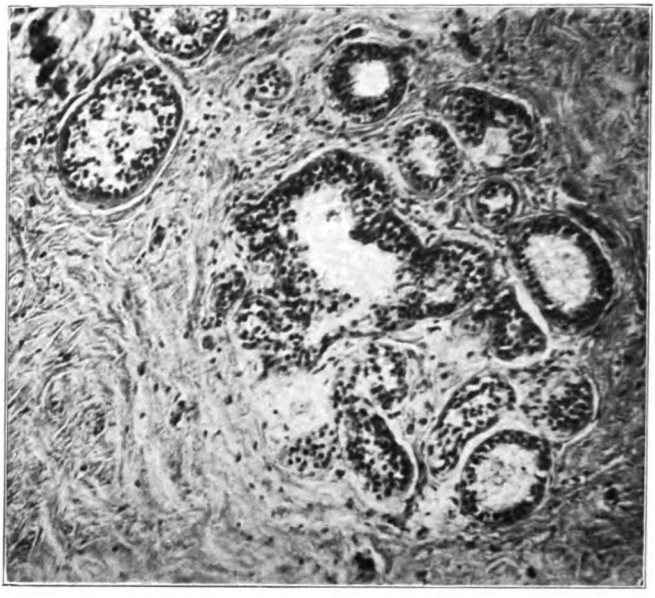

Fig. 3.—Breast; acini showing secondary hyperplasia and confusion of line of demarcation between acinus and stroma (MacCarty).

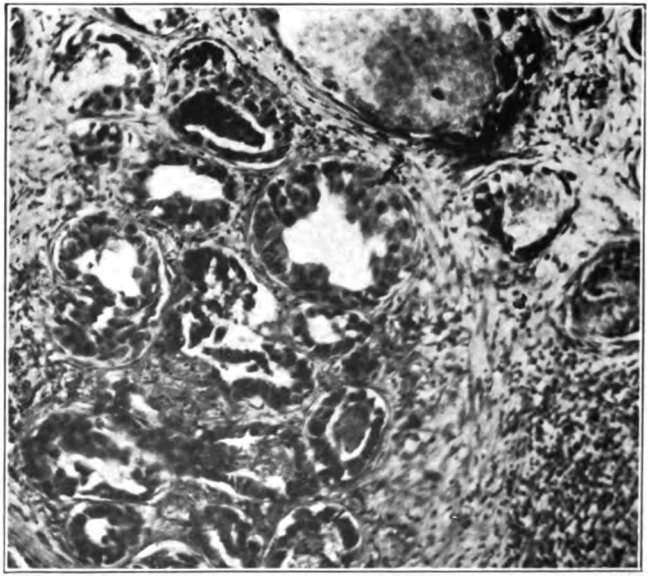

Fig. 4.—Breast; acini showing secondary epithelial hyperplasia (MacCarty).

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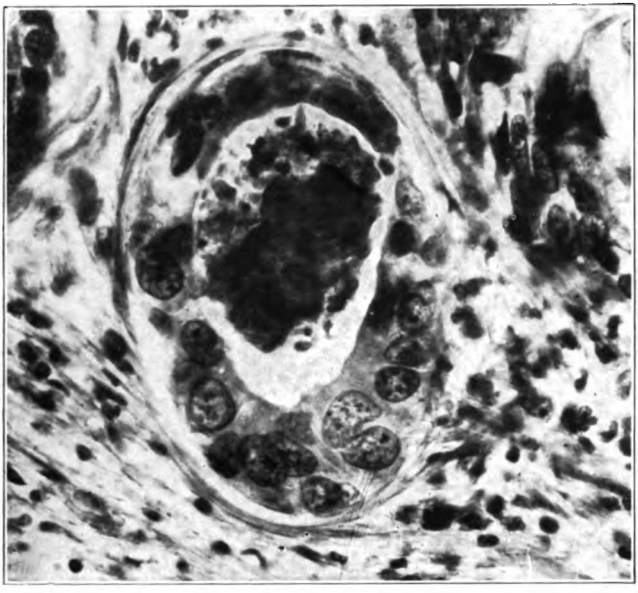

Fig. 5.—Breast; acinus showing secondary epithelial hyperplasia with irregularity of nuclei (MacCarty).

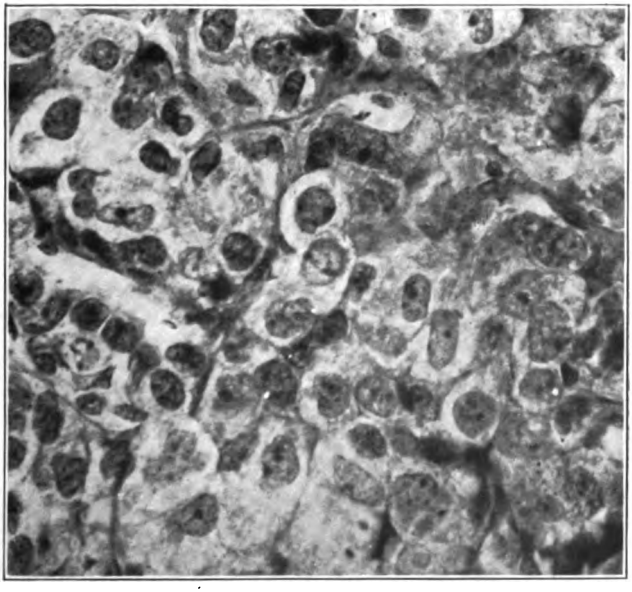

Fig. 6.—Breast; tertiary epithelial hyperplasia, showing irregularities of nuclei—cancer (MacCarty).

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Fig. 7.—Normal prostatic epithelium.

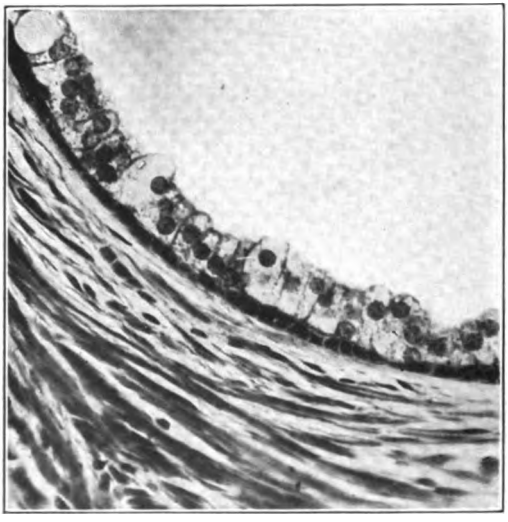

Fig. 8.—Normal prostatic epithelium.

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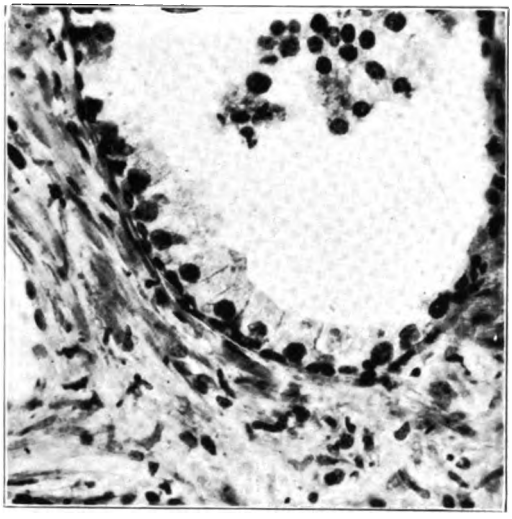

Fig. 9.—Normal prostatic epithelium.

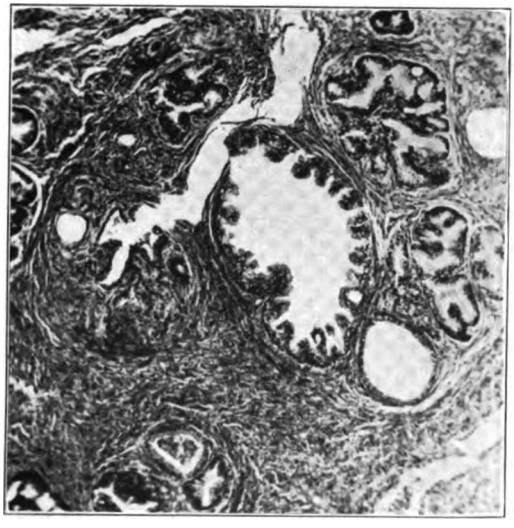

Fig. 10.—Normal prostatic epithelium, proliferating and projecting in masses in the lumen of the acinus.

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Fig. 11.—Normal prostatic epithelium, proliferating and projecting in masses in the lumen of the acinus.

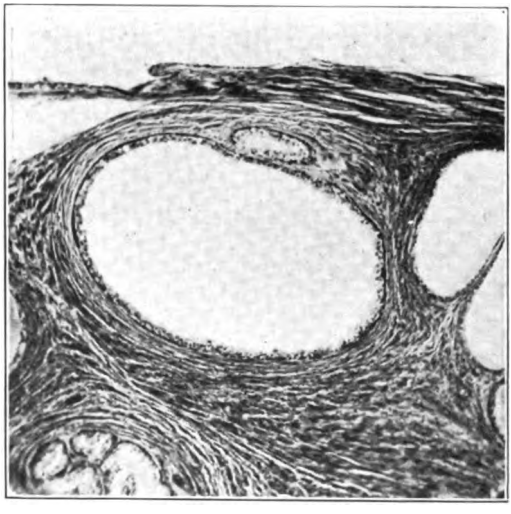

Fig. 12.—Cystic acini, showing two rows of cells, inner and outer row, similar to pictures seen in breast in primary hyperplasia.

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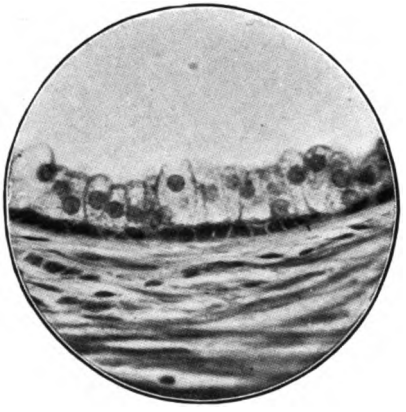

Fig. 13.—Cystic acini, showing two rows of cells, inner and outer row, similar to pictures seen in breast in primary hyperplasia.

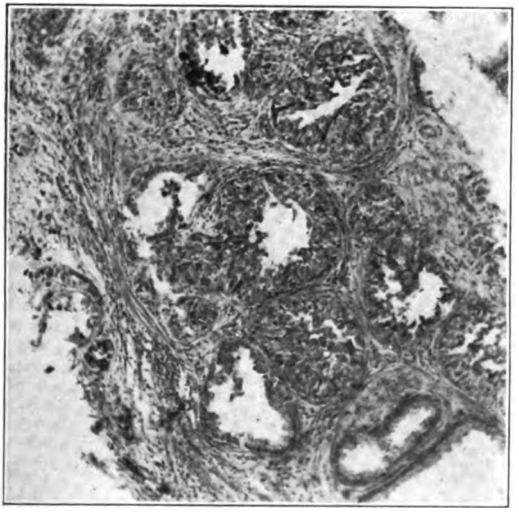

Fig. 14.—Normal acini, acini with hyperplasia of cells of outer row (secondary hyperplasia) and invasion of tissue by cells, which are morphologically indistinguishable from the intra-acinic hyperplastic cells of the outer row.

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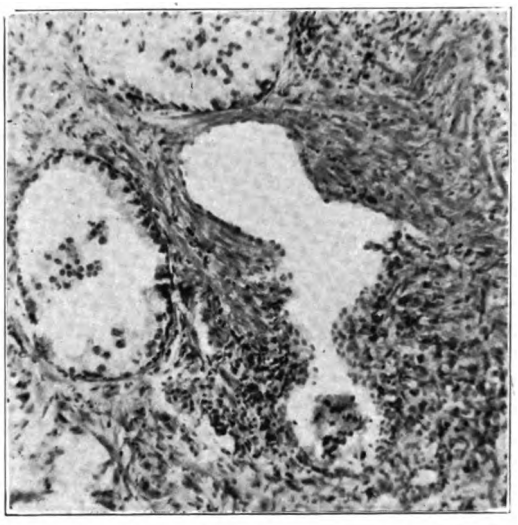

Fig. 15.—Normal epithelium in acinus, acinus with exfoliation of differentiated cells, acinus with some exfoliated differentiated cells and invasion (?) of stroma by hyperplastic cells of the outer row.

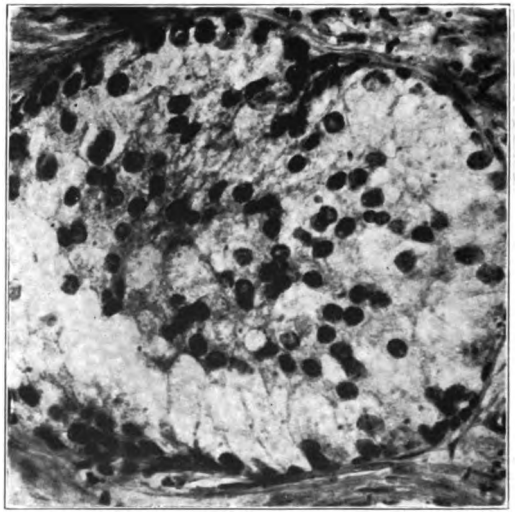

Fig. 16.—High power of acinus in Figure 15, showing both exfoliated differentiated cells and hyperplastic cells of the outer row.

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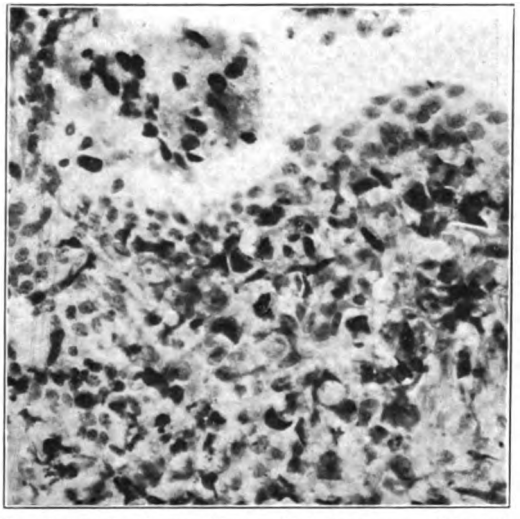

Fig. 17.—Acinus in Figure 15, in which there are exfoliation of differentiated cells, hyperplastic cells of the outer row and immediate peri-acinic invasion of stroma by hyperplastic cells of the outer row.

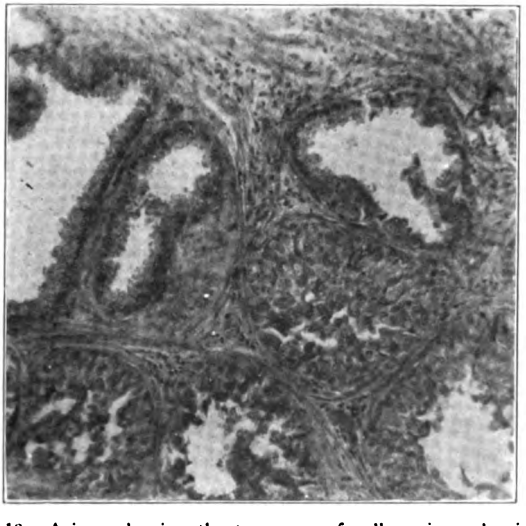

Fig. 18.—Acinus showing the two rows of cells, acinus showing the hyperplastic cells of outer row, completely filling the lumen.

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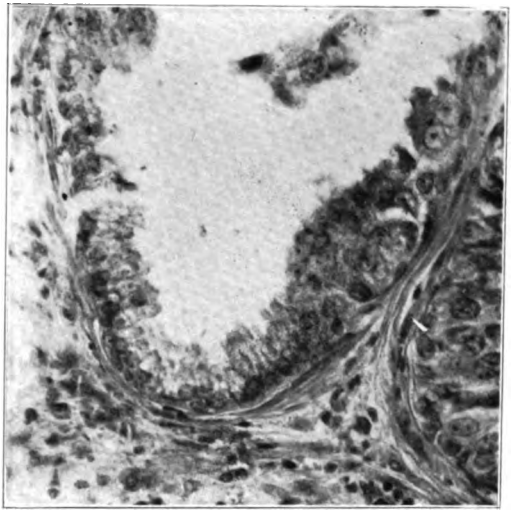

Fig. 19.—Acinus with primary hyperplasia on the left and secondary hyperplasia on the right and part of another acinus with secondary hyperplasia.

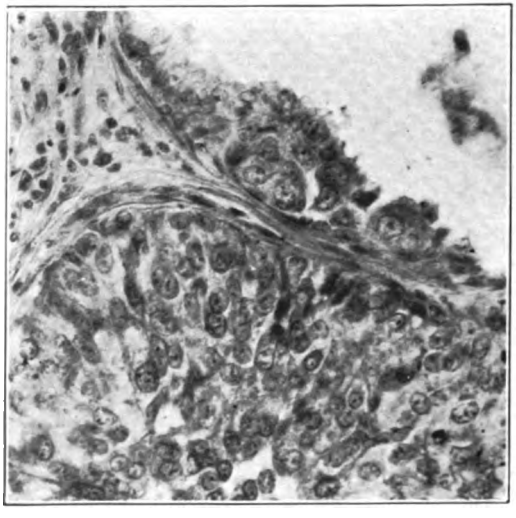

Fig. 20.—Same as Figure 19, showing in upper acinus primary and secondary hyperplasia; in lower acinus, secondary hyperplasia.

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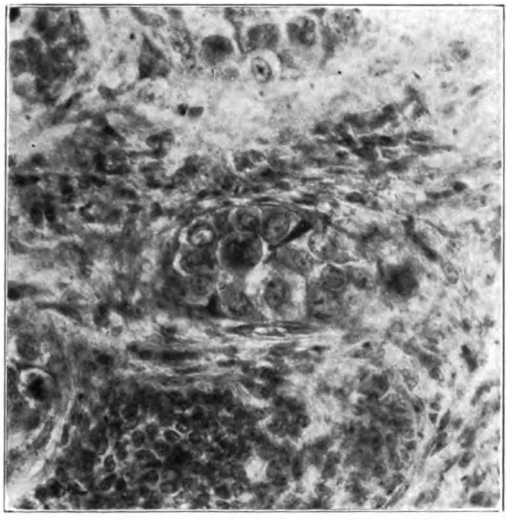

Fig. 21.—Same slide as Figures 18, 19 and 20, showing hyperplastic epithelial cells in the stroma, probably a lymphatic space (tertiary epithelial hyperplasia, or cancer). The cells are morphologically indistinguishable from the intra-acinic hyperplastic cells of the outer row (secondary hyperplasia).

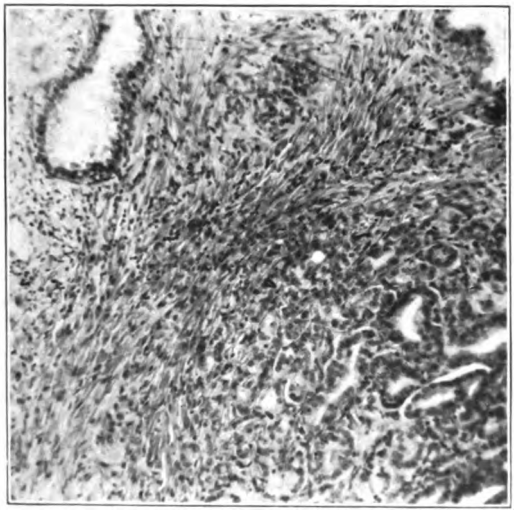

Fig. 22.—An apparently normal acinus above, to the right, and adenocarcinoma below, to the left, with fibrous band between.

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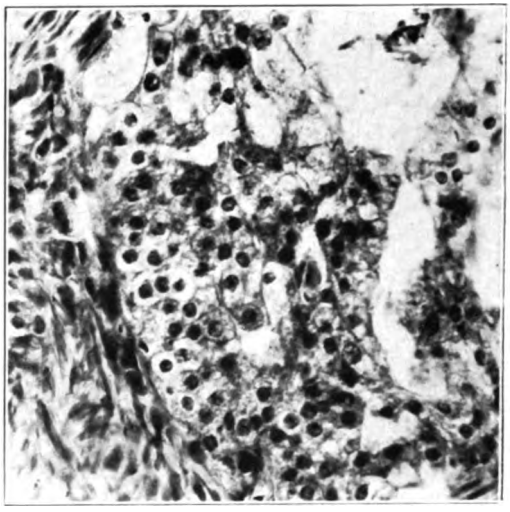

Fig. 23.—Tertiary hyperplasia—cancer.

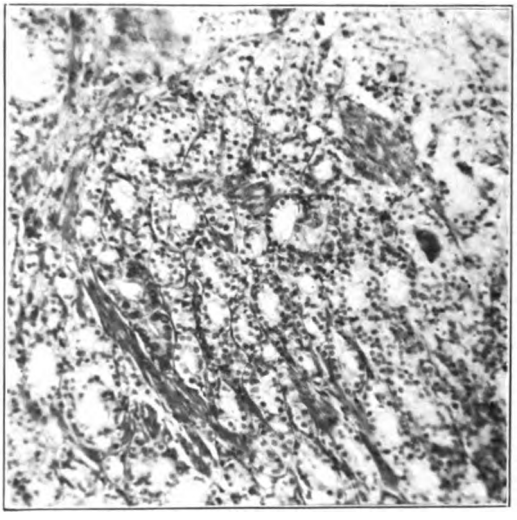

Fig. 24.—Adenocarcinoma.

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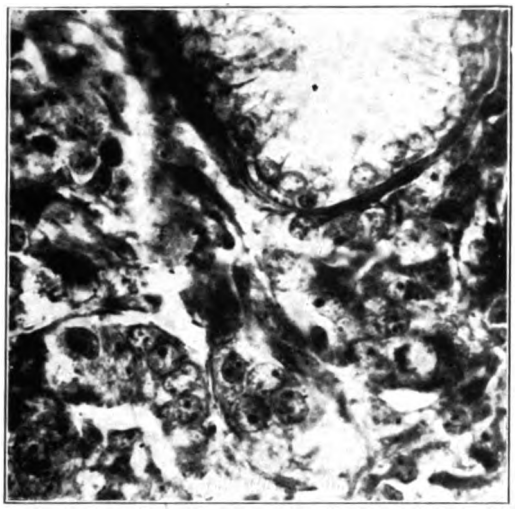

Fig. 25.—High power of adenocarcinoma.

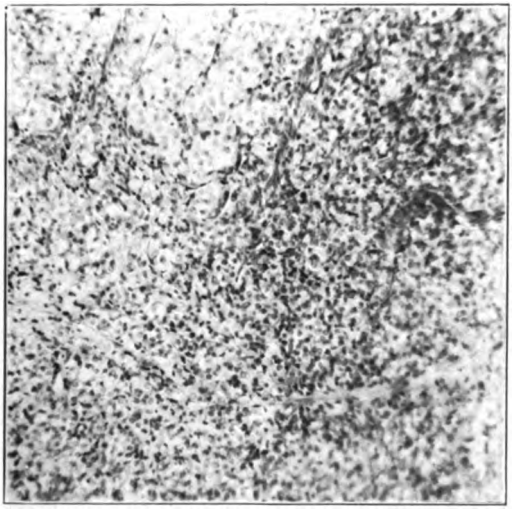

Fig. 26.—Medullary and diffuse carcinoma.

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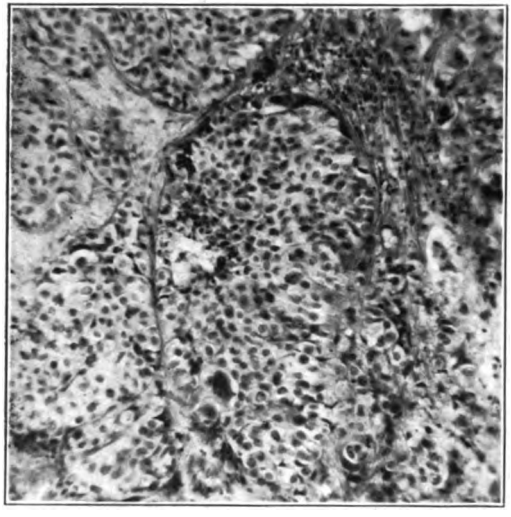

Fig. 27.—Medullary carcinoma.

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to the process of cancerous development; that this is essentially a process of epithelial hyperplasia, involving the above-described undifferentiated cells, in a state of reaction to an abnormal irritant. Furthermore, for the pathogenesis of cancer it would not be necessary to assume the theory of "embryonic rests."

Frequency.—Statistics indicate that we are indebted to the investigators of the past half century for the existing knowledge of cancer of the prostate. The first useful statistics were compiled by Thompson[4] (1854) who cited 18 cases from the English and the French literature. Wyss[4] (1866) reported 28 cases, including Thompson's; Socin[4] (1875), 50 cases; Kapuste[4] (1885), 54 cases; Engelbach[4] (1888), 144 cases; Wolff[5] (1899), 110 cases. Since 1902, numerous reports of malignant prostatic conditions have been contributed. It is the statistics of the more recent writers, however, which, although varying within somewhat wide limits, afford us the nearest approximation to the truth with regard to the frequency with which cancer occurs in the prostate.

In 500 cases of prostatic obstruction, Young[6] found cancer in 20 per cent. The same author states that about one out of every seven enlarged prostates after 50 years of age is cancerous. Albarran and Hallé noted 14 per cent.; Walker,[7] 16.5 per cent.; Wilson and McGrath[8] (1911), 468 reexamined prostatic specimens removed at operation, 15.5 per cent.; Freyer[9] (1914), 1,276 cases of enlarged prostate, 13.3 per cent. In the present series of 716 specimens (including the 468 cases reported by Wilson and McGrath in 1911), 12.28 per cent.† are cancer.

4. Cited by von Frisch, Der Krankheiten der Prostata, Ed. 2, 1910.
5. Wolff: Deutsch. Ztschr. f. Chir., July, 1899, lii, Hefte 3 and 4.
6. Young, Hugh, H.: Ann. Surg., 1909, l, 1232; The Early Diagnosis and Radical Cure of Carcinoma of the Prostate, THE JOURNAL A. M. A., March 10, 1906, p. 699.
7. Walker: Lancet, April 11, 1908, p. 1054.
8. Wilson and McGrath: Surg., Gynec. and Obst., December, 1911, p. 647.
9. Freyer: Arch. Internat. de chir., 1914, vi, No. 4.

† In the last 248 cases of this series only the specimens already diagnosed cancer were studied. It is probable that reexamination of the remaining specimens will increase the percentage of malignancy.

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Age.—One patient was between the ages of 40 and 50. Seventy-eight per cent. were between 60 and 80. Other investigators have found about 68 per cent. between the ages of 50 and 70. Wolff reports six patients under 40; one, 29 years of age.

Mode of Involvement.—Cancer of the prostate is primary in the great majority of cases. The gland is involved secondarily by adjacent cancer or through metastasis by way of the blood-stream. Rectal ulceration is the most frequent source of secondary prostatic cancer from contiguous structures. About one-half the cases of secondary involvement are metastatic from remote organs.

Site of Origin.—The most commonly ascribed site of the original focus in primary cancer of the prostate is the posterior lobe; but its presence in the lateral and middle lobes have been noted by some authors.

Growth.—The cancerous process has been observed to involve the entire prostate symmetrically and rapidly, but the consensus of investigators' opinions is that, in by far the large proportion of cases, cancer of this gland is limited, occurring as one or several little nodules and of slow growth. Consequently, gross serial sections of the specimen and microscopic examination of every doubtful area are essential for the diagnosis in the great majority of cases. Failure to appreciate this point, also neglect to examine the posterior lobe, are the probable cause of statistics with low percentage of cancer, likewise of inadequate treatment and erroneous prognosis.

Extension.—In the extension of prostatic cancer the fascia of Desnonvillers, also the fibrous capsules, both of the prostate itself, and of hypertrophied spheroids, lobules or lobes, are accredited with an essentially important rôle. Young emphasizes this point. Cuneo and Veau[10] have shown that the fascia of Desnon-

10. Cuneo and Veau, cited by Bryan: Lancet-Clinic, April 20, 1912, p. 433.

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villers is a prolongation downward of the peritoneum in fetal life, which projecting in a plane between the prostate and the seminal vesicles in front, and the rectum behind, by virtue of its reduplication doubly inhibits the spread of cancerous cells posteriorly. Consequently, ulceration of the rectum is infrequently seen and periprostatic structures are quite effectively guarded. The tendency, then, of the cancerous process is to extend upward to the region of the ejaculatory ducts, between the fascia of Desnonvillers posteriorly and the trigone anteriorly. Young maintains that the mucosa and submucosa of both urethra and bladder are very resistant to the malignant invasion. On the other hand, von Frisch[11] notes extensive involvement of both these structures. The seminal vesicles are frequently involved, and the growth may extend along the ureters, causing complete obstruction, even rupture. Extension upward for a long distance within the vasa deferentia, without apparent involvement of the outer walls, may occur (Young). Cases noted by von Frisch were: Growth into rectum with subsequent extensive involvement of the sacrum. Growth through the obturator foramen and between the adductors of the thigh. Once the capsule is permeated, the cancerous process spreads extensively.

Metastasis.—This occurs in bones, lymphatic glands and internal organs. Important to be emphasized is the fact that, whereas the primary cancer of the prostate is usually small, metastases from it are commonly extensive. The most frequent sites are the skeletal bones and the lymphatic glands. Visceral metastasis is comparatively infrequent, probably occurring in about 25 per cent. Kaufmann[12] remarks the infrequency of glandular metastasis from prostatic cancer compared with that from cancer of other structures. In 100 collected cases this author found involvement

11. Von Frisch: Die Krankheiten der Prostata, Ed. 2, 1910.
12. Kaufmann: Lehrbuch der speciellen pathologischen Anatomie, Berlin, 1901.

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of the pelvic glands 27 times; iliac, 24; inguinal, 16. Young, whose figures agree with those of Kaufmann as to the involvement of the pelvic glands, says: "It shows the fact that one should not expect enlarged glands before making a diagnosis of carcinoma of the prostate." Blumer[13] states that cancer of the prostate gives rise to metastasis in bones in a much larger proportion of cases than any other form of cancer — probably in two-thirds of patients in whom it is allowed to run an unobstructed course. Von Recklinghausen[14] emphasizes the fact that the cancerous focus in the prostate is, in comparison with the advanced foci in the bone, small and insignificant. The author directs attention to the analogy between metastasis in bone from a small nodule of cancer in the female breast and that from cancer of the prostate, and gives warning to suspect these sources in case of severe pain in the spinal column without evident cause. Blumer advises similarly in case of spastic paraplegia — involvement of the spine — and apparent osseous tumors in flat and long bones. In the thyroid, likewise, certain forms of malignant tumors occur which present entirely analogous relations, and such growths may be so small as to escape attention (von Eiselsberg[15]). The metastasis in bone is more or less general, its transmission is vascular rather than lymphatic and nearly always originates in the medulla—rarely beneath the periosteum (Blumer).

SUMMARY

1. An analogy exists between the microscopic fields of epithelial changes observed in cancer of the prostate and those of the mammary gland, as presented by MacCarty.

2. In the undoubted histologic picture of cancer, both organs present in the stroma migratory epithelial

13. Blumer: Johns Hopkins Hosp. Bull., July, 1909, p. 200.
14. Von Recklinghausen Festschr. zu Virchow 71. Geburtstag, Berlin, 1891.
15. Von Eiselsberg: Arch. f. klin. Chir. (Langenbeck), xlvi, 1893.

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cells which are morphologically indistinguishable from the hyperplastic undifferentiated cells within the acinus.

3. According to the studies of MacCarty, these intra-acinic, hyperplastic, undifferentiated cells represent the precursors of the specific functional cells of the gland; consequently, the positively cancerous cells are the direct, immediate descendants of those which become the differentiated cells under normal conditions.

4. This analogy between the prostate and the mammary gland being accepted, further support is added to MacCarty's deductions, drawn from extensive studies of the breast. And, if adequately prolonged and comprehensive investigations in the future confirm the present view of the latter author, then it may be stated of prostatic cancer, as well as of cancer in general, that the process is fundamentally one of epithelial hyperplasia of the above-described undifferentiated cells, in a state of reaction to an abnormal irritant; furthermore, that for the pathogenesis of cancer it would not be necessary to assume the theory of "prenatal or postnatal rests."

5. Cancer of the prostate is a comparatively frequent disease after 50 years of age.

6. The process usually begins in the posterior lobe and occurs more commonly as a small nodule or nodules of slow growth; consequently, *careful investigation of the posterior lobe, gross serial sections of the specimen, with microscopic examination of doubtful areas, are essential for diagnosis in the majority of cases.*

7. The fascial strata associated with the prostate seem to be quite effective barriers to the progress of the disease.

8. The tendency of extension of the cancerous process is upward to the region defined by the fascia of Desnonvillers posteriorly and the trigone anteriorly, and then beyond this area.

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9. Metastasis affects bones, lymphatic glands and internal organs. The cancerous focus in the prostate may be, and commonly is, insignificant, while the metastasis in bone is extensive. Similar relations have been observed with small cancerous nodules in the female breast and the thyroid. Manifestations in bone, with cause obscure, should direct attention to these observations.

10. Involvement of lymphatic glands is not a dependable factor in diagnosis, since metastasis may be present in the skeletal structure and these glands apparently unaffected.

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THE PINEAL GLAND IN RELATION TO SOMATIC, SEXUAL AND MENTAL DEVELOPMENT

CAREY PRATT McCORD, M.D.
DETROIT

From tumors involving the pineal gland, two distinct systems of symptoms and signs ensue, the neurologic and the metabolic. The neurologic manifestations arise from the encroachment of the neoplasm on the intracranial contents and are indications of changes in pressure, in placement and of destruction of tissue. Such changes are the consequence of pineal tumors at any age of the patient; but in pineal tumors appearing in prepuberal life a second group of changes arise, the metabolic. These metabolic alterations are referable to disturbance in the gland's secretory function. Apparently only in young males is this syndrome complete.[1] It consists of (1) early sexual development evidenced in the enlarged sex organs, pubic hair, general body hair, early changes in voice; (2) precocious mental development evidenced in the maturity of thought and speech, and (3) general body overgrowth to the extent that a child of 5 or 6 years may have the appearance of a child of 11 or 12.

A case reported by Machell[2] presents these changes in a striking manner. The patient was a boy less than 6 years old at the time of the publication. At the age of 5 months there was pubic hair, erections at 17 months, emissions at 30 months. The patient's weight was 7½ pounds above normal at 4 months of age, 12 pounds in excess at 8 months, 20 pounds in excess at 3 years. When the patient was 44 months old, his

1. For review of clinical cases see Bailey, Pearce, and Jelliffe, Smith Ely: Tumors of the Pineal Body, Arch. Int. Med., December, 1911, p. 851. For Physiology and Anatomy, see Vincent Swale: Internal Secretion and Ductless Glands, 1912.
2. Abstract taken from Medical Chronicle, 1912, lvii, 154.

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height was 8½ inches above normal for a child of that age. At 48 months the circumference of the head was over 2 inches in excess of normal. The voice was a deep bass. Mental precocity was very marked and the general bearing and language was that of a much older person.

On account of the difficulties attending experimentation on this vestigial organ, the clinical findings with subsequent necropsy records have been the prime factor in the formulation of the prevailing ideas as to this gland's functions. The conception of this gland's function, however, has in part been developed from laboratory studies, notably from the results concomitant to the extirpation of the organ. This has been attempted frequently, but the situation of the gland is such that in the greater number of instances death followed the operative procedure, from hemorrhage or injury to the vermis or the occipital lobes. By operating on a large number of animals some workers have had a few animals survive. No changes attended the removal of the gland by Biedl,[3] Dandy[4] and Exner and Boese,[5] but Foa[6] ablating the gland in chicks and Sarteschi[7] in young rabbits and puppies report the production of the precocious macrogenitosomatic syndrome.

The publications growing out of these several clinical and laboratory studies have given rise to conceptions of this organ and its functions that may be thus epitomized:

The pineal body (epiphysis cerebri), probably the remains of a parietal eye, is situated just beneath the splenium of the corpus callosum, resting on the anterior quadrigeminate bodies, and is attached by its base to the habenular commissure. The gland varies in size, shape and pigmentation and does not stand in any proportional relation to the size of the brain or size of the body. The pineal body contains glandular elements, but these are few and not well defined. The greatest

3. Biedl: Innere Sekretion, 1910.
4. Dandy, cited by Cushing: Pituitary Gland and Its Disorders, p. 283.
5. Exner and Boese: Deutsch. Ztschr. f. Chir., 1910, cvii, 182.
6. Foa: Arch. Ital. de Biol., 1912, lvii, 233.
7. Sarteschi: Pathologia, 1913, p. 707.

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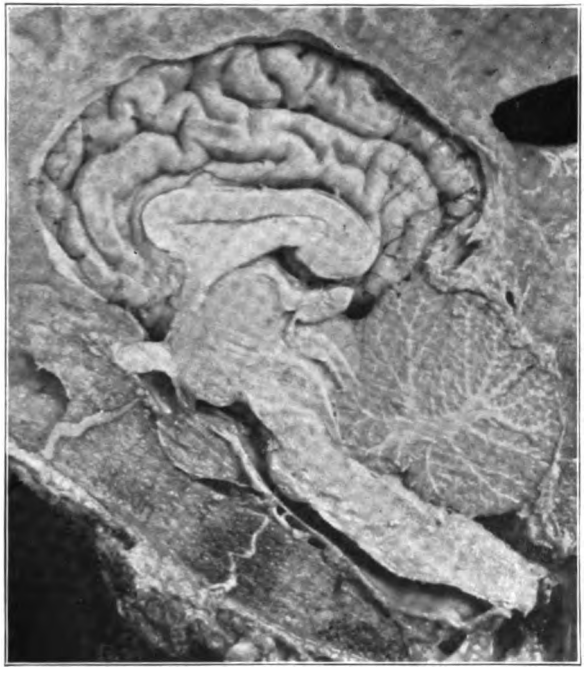

Fig. 1.—Position, relations and relative size of the pineal gland. Taken from beef 3 years of age.

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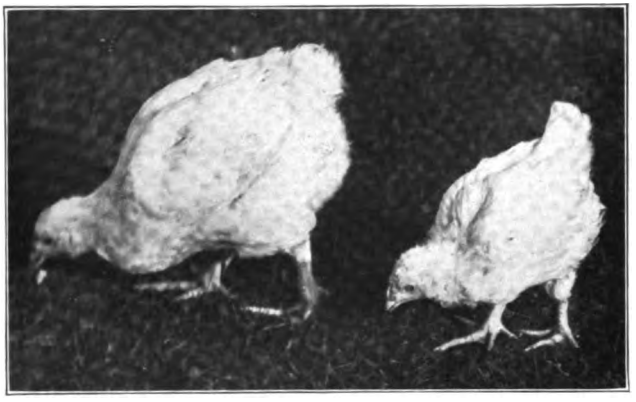

Fig. 2.—Effect on growth of feeding pineal-gland tissue to chicks. The larger chick was fed 10 mg. pineal tissue three times weekly.

Fig. 3.—Effect on growth of guinea-pigs produced by feeding pineal-gland tissue. Control pigs to left, test pigs to right.

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postnatal development is in the first years of life, and as far as is known the gland is only functionally active in the prepuberal life. On the assumption that tumors destroy the gland and deprive the body of the substances generated by it, the function of the gland is generally described as retarding and holding in abeyance too rapid development in childhood, of the body, mind and sexual characteristics. When precocity in development appears in conjunction with pineal tumors, it is attributed to lack of glandular secretion.

Below are recorded some findings from experimental work that are entirely in accord with the results obtained by Dana and Berkeley.[8] Contrary to the view that precocious development is due to pineal deficiency (hypopinealism) the same precocity in development has been brought about by the oral administration of pineal gland tissue.

EXPERIMENTAL DATA

The general plan of work entailed in this study has been to feed to very young animals minute quantities of pineal tissue and to record the weight changes, sexual differences, and in the case of dogs increased mentality, over control animals maintained under otherwise identical conditions. One hundred and ten guinea-pigs, eighteen puppies, fourteen adult dogs and sixteen chicks have been under observation.

Fresh pineal glands from cattle have been employed. These were in part from veal, in part from young adults approximating 3 years, and in part from the general run of cattle from the abattoirs. The glands averaged in weight (on weighing sixty glands) 2.14 grains. There were in the many thousand glands making up the several pounds that have been used, marked variations in size, shape and melanopigment. This pigment, which was present in a high percentage of glands, involves chiefly the encapsulating tissue.

For feeding experiments the glands were prepared for permanent use in the following manner: The fresh glands were rinsed free of blood and stripped of adherent tissue. They were ground to a fine paste in

8. Dana and Berkeley: Med. Rec., N. Y., 1913, lxxxiii, 835.

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the Latapie grinder, and without drying, the paste was admixed with milk-sugar in such quantity that ½ grain milk-sugar represented 10 mg. pineal tissue. The mass was made into ½-grain tablets and quickly dried at room temperature.

Early in the work it was apparent that the more striking results were being obtained from the animals fed with pineal substance from cattle not having reached adult life. Efforts were then made to establish quantitatively the activity and the identity of the various experimental lots of pineal preparations by employing the methods commonly used in testing the activity of endocrinous derivatives. Although certain cardiovascular changes regularly follow the intravenous administration of pineal extracts to dogs, the extent of these changes is not a measure of the activity of the gland as a stimulator of growth.

On the hypothesis that precocious development is due to hypopinealism, the first work was done in anticipation that feeding would retard development and prolong the presexual life. This was begun on two chicks incubated in the laboratory. Beginning at the age of two days one was fed 10 mg. veal pineal tissue, three times weekly, the other (and in all cases of controls) was fed a blank tablet of milk-sugar. The difference in growth should be noted (Table 1).

TABLE 1.—RESULTS OF FEEDING PINEAL GLAND TO CHICKS

	Third Week, gm.	Sixth Week, gm.	Ninth Week, gm.	Twelfth Week, gm.
Pineal	219.5	557.7	895	925
Control	92.5	286.5	550	700
Difference	127.0	271.2	345	225

The striking disproportion in size and the marked skeletal overgrowth, making the large chick very awkward in his movements, soon made these chicks a laboratory curiosity, but the small number and the different sex did not justify any inference as to the

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influence of the pineal feeding. The results, however, were so striking that at once work was instituted in a more extensive way.

A lot of fifty guinea-pigs in the second week of life was selected and divided into test and control groups. The test pigs were fed daily 10 mg. veal pineal tissue. The controls were fed a ½-grain milk-sugar tablet. Other conditions for the two lots were identical. The results obtained are given in Table 2.

TABLE 2.—RESULTS OF FEEDING PINEAL GLAND TO YOUNG GUINEA-PIGS

Control, 25 Pigs Average Initial Weight, 201.2 gm. Age, 2 Weeks			Pineal, 25 Pigs Average Initial Weight, 204.3 gm. Age, 2 Weeks		
Weeks	Aver. Wt. gm.	Gain gm.	Weeks	Aver. Wt. gm.	Gain gm.
1	198.7	—2.5	1	213.2	8.9
2	220	21.3	2	236.8	23.6
3	226.8	6.8	3	247.6	10.8
4	251	24.2	4	273.2	25.6
5	265.3	14.3	5	299.9	26.7
6	265.7	0.4	6	311.1	11.2
7	292.2	26.5	7	338	26.9
8	306	13.8	8	361.4	23.4
9	316.3	10.8	9	365.5	4.1
10	356.5	40.3	10	410.8	45.3
Average weight at end of tenth week.....356.6 gm. Average initial weight...201.2 gm. Average gain.....155.4 gm. Gain, per cent..... 77.0			Average weight at end of tenth week.....410.8 gm. Average initial weight...204.3 gm. Average gain.....206.5 gm. Gain, per cent.....100.0		

Excess gain of pineals over controls, 23 per cent.

This excess in weight of pineal-fed guinea-pigs over their controls is a symmetrical overgrowth. There is some increased adipose tissue, but this is generally distributed and not localized in any one region of the body. At no time has it been possible to continue this excessive growth above normal adult size. As the animals approach adult size the pineal feeding is less effective and after full maturity is attained is without effect. There has been no tendency to gigantism.

As a step toward determining the metabolic differences, quantitative urinalyses were made on twenty-

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four-hour composite samples of urine from the two groups.[9] With proper regard for the numerous other factors that might vitiate the results obtained from urinalyses, there is a suggestive difference in the two urines as may be seen in the summary given in Table 3.

TABLE 3.—SUMMARY OF ANALYSIS OF URINES

	Pineal	Control
Total volume collected (24 hours)	187 c.c.	160 c.c.
Specific gravity (15.5 C.)	1.016	1.019
	Per Cent.	Per Cent.
Total solids	2.51	3.02
Water	97.49	96.98
Total mineral matter	1.21	1.37
Total nitrogen	0.628	0.872
Total creatinin	0.018	0.025
Phosphoric acid (as P_2O_5)	0.065	0.043
Calcium	0.016	0.018
Magnesium	0.020	0.018
Alkalinity (as Na_2CO_3)	0.053	0.037

Chicks.—A lot of fourteen chicks was secured at the age of one week. At so early an age sex could not be determined and the lot was divided into test and control groups without knowledge as to grouping by sex. The test chicks were placed on veal pineal tissue for one week with a resultant greater growth than controls. For the next four weeks they were fed pineal tissue from old cattle, without gaining. On being placed on the original veal preparation the test chicks again grew in excess over controls.

Dogs.—The eighteen dogs employed represent four litters. Infection of these puppies with distemper interfered seriously with the weight charts of these animals. So long as infections could be kept out of the animal quarters, the test animals outgrew the controls, but the wasting from infections interfered seriously with average results over prolonged periods. It was in connection with puppies that some differences in intelligence were observed. No great import is attached to these observations, but it was noted that the pineal-fed dogs were about one month ahead of the others in their habits. They were the first to learn

9. I am under obligations to Mr. Lewis Davis for these analyses.

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to lap milk, the first to respond to a call, the first to be able to find their way back to the kennel. When work on these animals was discontinued, only the pineal-fed animals were in demand as pets, and those choosing them did so without any knowledge as to differences in feeding. Of much more importance as regards increased intelligence are the favorable results reported by Berkeley,[10] who administered pineal tissue to mentally defective children and performed Binet tests as a criterion of mental advancement.

Precocious Sexual Development.—A group of forty-eight guinea-pigs was divided into test and control lots. There was an equal number of males and females in each lot, but the males and females were separated. The test pigs were fed veal pineal tissue in 10 mg. amounts daily. Feeding was begun when the animals were 2 weeks old and continued for nine weeks. The males and females of each group were then placed together in breeding-pens. As a measure of any difference in sexual development it was thought desirable to note the date of birth of young in the two lots. As the end of the approximate gestation period approached, these animals were observed as to the date of giving birth to young. All except two of the pineal-fed pigs gave birth to young before the first of the controls.† Fourteen days elapsed between the birth of young of the first pineal-fed pig and the first control pig. In all cases the young were normal and in no wise different from any other young pigs.

COMMENT AND SUMMARY

In the foregoing records of experiments, it may be observed that some of the changes generally attributed to deficiency of pineal secretion may be produced by

10. Berkeley: Med. Rec., New York, 1914, lxxxv, 85, 513.

† In a second series of fifty guinea-pigs observed as to sexual differences, the males and females have been kept together from birth. Some of the females to whom pineal gland tissue has been fed have already given birth to young and many others are within the last ten days of gestation. With the exception of one pig, the control animals evince no signs of pregnancy.

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supplying an added amount of pineal substance. In an effort to reduce to rationality these identical findings derived from two opposing sources, the so-called destructive neoplasms of the pineal gland on the one hand, and the feeding of the gland on the other, there arise two possibilities: First, this syndrome may appear from disrupting the general endocrinous balance from either increasing or decreasing the amount of pineal secretion available for the body's use. These secondary changes in the other endocrinous glands are now in the course of investigation. Second, the cells of the neoplasms involving the pineal gland may retain some of the metabolic and other functional characteristics of the normal pineal cell from which they were derived, and the peculiar body, sexual and mental changes in patients with such tumors are all manifestations of increased rather than decreased pineal activity. One of the most frequently occurring lesions of the pineal is the adenoma, and there is abundant evidence that at times cells of adenoma functionate after the manner of the cells from which they arise. In adenoma of the liver in cases reported by Weber,[11] Rolleston,[12] Wheeler[13] and Ribbert,[14] distinct bile secretion by tumor-cells has been pointed out. Ribbert established that the bile present in such tumors was not the bile of icterus from necrotic liver-tissue by demonstrating that the scirrhous encapsulating tissue was free from bile-stain and that the bile was confined to the liver-like cells of the active tumor. In at least one case, a metastasis in the lung from the liver secreted bile. In this connection it is significant that the functionating glandular cells of typical thyroid structure have been found in thyroid metastases in bone-tissue. Furthermore, in myeloma of the bone-marrow the cytoplasm[15] of the tumor-cells contain the

11. Weber: Proc. Roy. Soc. Med. (Path. Sec.), 1910, iii, 147.
12. Rolleston: Diseases of the Liver, 1905.
13. Wheeler: Guy's Hosp. Rep., 1909, lxiii, 225.
14. Ribbert: Deutsch med. Wchnschr., 1909, xxxv, 1607.
15. Weber and Ledingham: Proc. Roy. Soc. Med. (Path. Sec.), 1909, ii, 206.

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granules that characterize normal myelocytes, that is, the tumor to a certain extent assumes the function of the bone-marrow. All considered, it is perceived that functional activity of tumor-cells is *not* infrequent. Germane to the present contention is the statement by Hinds Howell[16] in describing the characteristics of the cells of the pineal tumors of his three cases. He says:

> A noteworthy feature is the similarity of these tumor-cells in many instances to those of the normal pineal gland.

The results of this work lead me to the conclusion that the administration of minute quantities of pineal tissue from young animals to young animals stimulates rapid growth of the body, but not beyond normal size. Also there are less well-established indications of precocity of mental and sexual development.

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16. Howell, Hinds: Proc. Roy. Soc. Med. (Neurolog. Sec.), 1910, iii, 65.

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IMMUNITY IN TUBERCULOSIS

FURTHER EXPERIMENTS *

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COLORADO SPRINGS, COLO.

In 1911, experiments were reported[1] on twelve monkeys. The subsequent history of those not killed at that time follows:

MONKEY 1.—Control. Jan. 19, 1912. The monkey had not become spontaneously infected since its purchase, June 8, 1910.

Oct. 14, 1912: Intradermal tuberculin test 0.1 mg. O. T. Result negative.

Dec. 1, 1912: Animal has refused to eat for three days. Found dead. Necropsy, no trace of tuberculosis anywhere.

MONKEY 2.—Received 25 virulent human tubercle bacilli by subcutaneous injection. June 9, 1910, thirty-five bacilli of this culture had infected a guinea-pig (Barber culture). June 7, 1911, killed. No evidence of tubercle anywhere. Lymph-nodes from various parts ground up and injected into guinea-pig without producing infection.

MONKEY 3.—Received 100 virulent human tubercle bacilli by subcutaneous injection, of same culture, June 8, 1910.

Sept. 6, 1911: Conditions resembling elephantiasis had developed. Animal killed. Lower half of vulva, buttocks, tail, legs, showed dense infiltration of skin about ½ inch in thickness. The swollen parts were soft but firm. Edematous, not reddened. All organs normal. No signs of tuberculosis anywhere. Pieces of swollen area, lymph-nodes from various parts ground up and injected into guinea-pig without infection resulting.

MONKEY 4.—Received 200 human tubercle bacilli of same culture by subcutaneous injection, June 8, 1910.

Dec. 27, 1911: Killed. No evidence anywhere of tuberculosis.

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* This work was assisted by a grant from the Committee on Scientific Research of the American Medical Association.

1. Webb, G. B., and Williams, W. W.: Immunity in Tuberculosis, THE JOURNAL A. M. A., Oct. 28, 1911, p. 1431.

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MONKEY 5.—Received in increasing doses, beginning with 1 bacillus, 3, 5 bacilli, etc., a total of 8,369 virulent human tubercle bacilli from June 8, 1910, to Feb. 3, 1911.

Dec. 21, 1911: Killed. No evidence anywhere of tuberculosis.

MONKEY 6.—Received similar inoculations to Monkey 5.

June 24, 1912: Second intradermal tuberculin test with 3.8 mg. O. T. Result negative.

Oct. 8, 1913: Third intradermal tuberculin test with 1 mg. O. T. Result negative.

Oct. 12, 1913: Found dead in cage. Necropsy. Scattered tubercles in left lung. Lower lobes of right lung solidified caseous areas and adherent to chest wall. Spleen badly infected. Bronchial glands contain pus. Original culture recovered from this monkey.

MONKEY 7.—Received in increasing doses beginning with 1 bacillus, 5, 10, etc., a total of 417,961 virulent human tubercle bacilli from June 8, 1910, to Feb. 3, 1911.

Dec. 7, 1912: Has refused to eat for a few days. Found dead. Necropsy: No trace of tuberculosis in any organ.

COMMENT.—Monkeys 1 and 7 were kept in the same cage. The culture given them for a test of immunity had originally infected a guinea-pig with only 125 bacilli. It was now found that 100,000 failed to infect a guinea-pig. It was later discovered that their keeper had given them oats a few days before their mysterious deaths.

MONKEY 8.—Inoculations given in increasing dose as in Monkey 7. Total bacilli received, 417,961. Necropsy June 8, 1911: No tuberculosis.

MONKEY 9.—Received Inoculations of virulent human tubercle bacilli beginning with 5 bacilli, 10, 15, etc., from June 8, 1910, to Feb. 3, 1911. Total, 15,720.

June 24, 1912: Intradermal tuberculin test, 3.8 mg. O. T. negative.

May 29, 1912: Weight, 4,703 gm.

Oct. 14, 1912: Weight, 5,754 gm.

Nov. 12, 1913: Subcutaneous test. See Chart 1.

June 15, 1914: Apparently perfectly well. Killed. Necropsy: No tuberculosis.

MONKEY 10.—Received inoculations of virulent human tubercle bacilli (Barber culture), beginning with 5 bacilli, 15, 30, etc., from June 8, 1910, to Feb. 3, 1911. Total, 37,805.

June 24, 1912: Intradermal tuberculin test, 3.8 mg. O. T. negative.

Jan. 3. 1913: Through the courtesy of Dr. Penrose, this monkey and also Monkey 12 were accepted by the Philadelphia Zoological Society. Both were retested with tuberculin before admission and found to be negative.

June 16, 1914: Dr. Herbert Fox reports: Both monkeys negative to tuberculin on admission. Not since tested. Both monkeys have failed a little in weight in last few months

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and Monkey 10 has slight cough. These monkeys have been possibly more exposed to the public than the others in the gardens. They have now been removed to outdoor cages.

MONKEY 11.—Control, killed. No tuberculosis.

MONKEY 12.—Received 50 virulent human tubercle bacilli at one dose, June 8, 1910.

May 22, 1911: Subcutaneous O. T. test negative.

May 21, 1912: Gave birth to dead baby.

June 24, 1912: Intradermal tuberculin test, 3.8 mg. O. T., negative.

Jan. 3, 1913: Forwarded to Philadelphia Zoological Gardens.

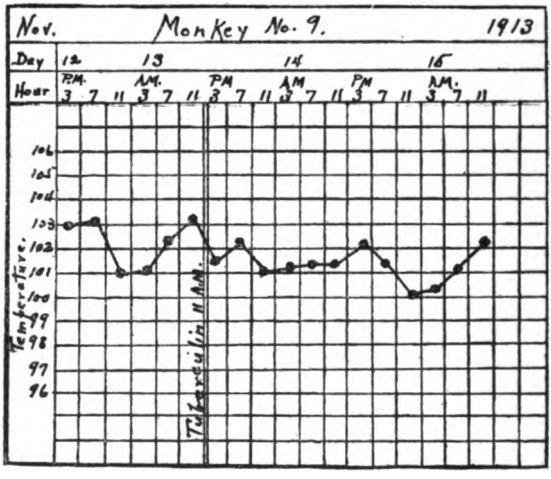

Chart 1.—Temperature chart of Monkey 9.

June 16, 1914: Report by Dr. Herbert Fox above: This animal we regarded as a control possibly to Monkey 10. Fifty virulent bacilli had not infected.

Twelve new monkeys (*Macacus rhesus*) were received June 17, 1912.

June 18, 1912: Conjunctival tests with 2 per cent. O. T. in right eye of each. All negative.

June 18, 1912: Simultaneously a von Pirquet test with pure O. T. was made in the neighborhood of the left nipple. All negative.

June 18, 1912: Differential blood-counts were made and the opportunity was taken to investigate any possible increase in their lymphocytes due to the altitude. Chart 2 depicts the results.

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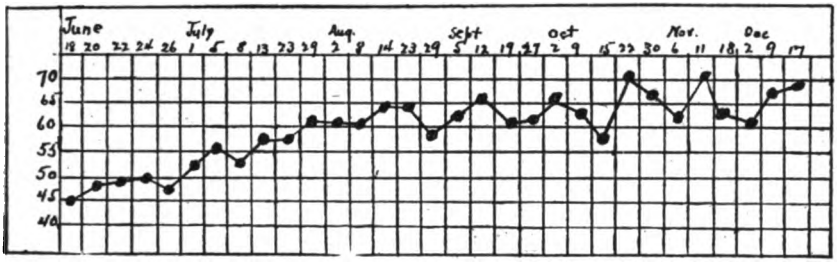

Chart 2.—Average mononuclear percentage of twelve monkeys.

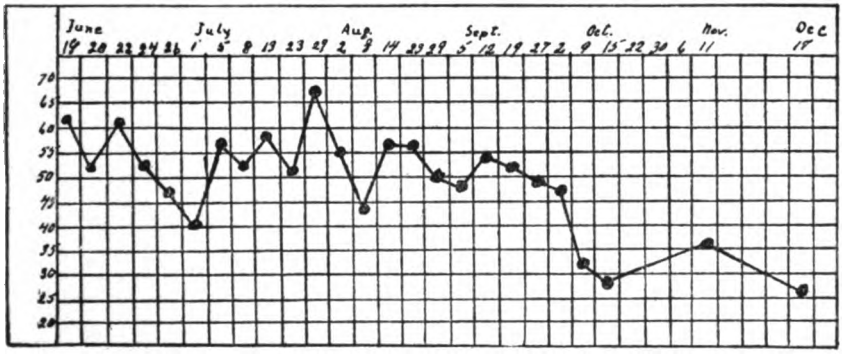

Chart 3.—Percentage of mononuclears, Monkey 14.

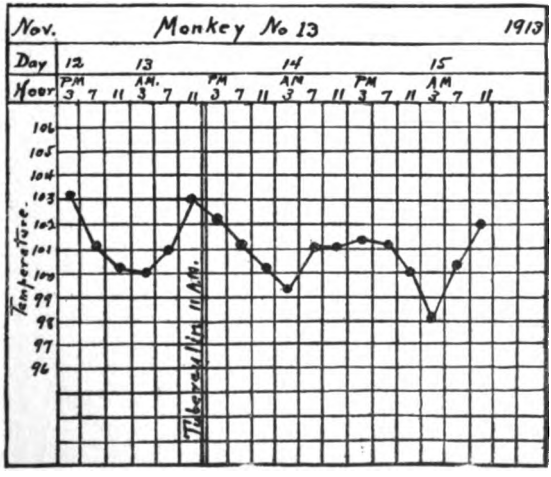

Chart 4.—Temperature chart of Monkey 13.

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Oct. 14, 1912: Monkeys 13 and 14 tested with O. T., 0.1 mg., intradermally. Monkey 14 reacted markedly with 150 per cent. increase. Chart 3 illustrates the change in the differential blood-picture in this monkey.

MONKEY 13—Having been in cage with Monkey 14, was not used for experiment. After two months exposure was killed.

Nov. 12, 1913: Subcutaneous tuberculin test appears negative (Chart 4).

Jan. 12, 1914: Lymphocytes had dropped from 63 to 14 per cent. Blood-platelets 596,000. White corpuscles 24,000.

Jan. 16, 1914: Evidently ill. Killed. No microscopic evidence of tuberculosis could be found except extensive adhesions about right lung. Entire base tightly bound down. Both adrenals very soft, mushy and necrotic. Smears showed no tubercle bacilli.

MONKEY 14—Oct. 14, 1912: Reacted to intradermal test as stated above.

Jan. 29, 1913: Killed. Peritoneum covered with tubercles. Spleen large and filled with caseous masses. Extensive lesions in liver. Scattered tubercles in lungs. This monkey was evidently infected on arrival or shortly after. The positive intradermal reaction was the first we had experienced in any monkey and the decisiveness convinced us that those reported in our former papers were probably not positive.

Through the courtesy of Dr. Lawrason Brown of Saranac, a culture (Brown C_4) was received from which Dr. Brown had been able to infect a guinea-pig with 20 bacilli. We wished to ascertain how many bacilli of this culture would, inoculated subcutaneously, infect a monkey.

Preliminary experiments were made on guinea-pigs with results mentioned below.

GUINEA-PIG 2.—Twenty-five bacilli inoculated into right nipple area.

Nov. 11, 1912: GUINEA-PIG 3: Several thousand bacilli inoculated into right nipple area.

GUINEA-PIG 4.—Twenty-five bacilli inoculated into right nipple area.

GUINEA-PIG 5.—Fifty bacilli inoculated into right nipple area.

GUINEA-PIG 6.—Fifty bacilli inoculated into right nipple area.

GUINEA-PIG 7.—One hundred bacilli inoculated into right nipple area.

GUINEA-PIG 8.—Ten bacilli inoculated into right nipple area.

All pigs became definitely infected and tuberculosis was confirmed by necropsies.

This culture, from which only *ten* bacilli could cause infection, was evidently more virulent than any we had before employed.

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Inoculations were made into guinea-pigs in increasing doses as the records indicate (Tables 1 and 2), but each pig finally developed tuberculosis.

TABLE 1.—INOCULATIONS INTO GUINEA-PIG 6

Date	Tubercle Bacilli	Date	Tubercle Bacilli
1/ 9/13	2	2/ 3/13	14
1/13/13	4	2/ 6/13	16
1/16/13	6	2/10/13	20
1/20/13	8	2/13/13	25
1/23/13	9	2/17/13	30
1/27/13	10	2/20/13	35
1/30/13	12	2/24/13	40

Feb. 27, 1913: Pig has local lump at inner side of left nipple.

TABLE 2.—INOCULATIONS INTO GUINEA-PIGS 30, 31 AND 32

Date	Tubercle Bacilli	Date	Tubercle Bacilli
5/19/13	R 2	6/23/13	L 12
5/26/13	R 4	6/30/13	L 14
6/ 2/13	L 6	7/ 7/13	R 16
6/ 9/13	L 8	7/10/13	R 20
6/16/13	L 10	7/21/13	R 20

Injections were made into right and left nipple areas according to designation R or L.

July 21, 1913: Probable infection of groin glands on left side.

Sept. 3, 1913: Definite infection of groin glands on left side.

MONKEY 15.—Jan. 20, 1913: Three hundred bacilli, Culture Brown C_4, inoculated just below left nipple.

March 4, 1913: Large local lesion at site of inoculation. Enlarged gland in left axilla. Intradermal tuberculin test + + (positive).

March 6, 1913: Killed. Local lesion and infected axillary gland. Otherwise no evidence of tuberculosis.

MONKEY 16.—July 1, 1912: Broken leg, discarded. Necropsy negative.

MONKEY 17.—Oct. 31, 1912: Prolapsed bowel, discarded. Necropsy: lobar pneumonia.

MONKEY 18.—Jan. 20, 1913: Four hundred bacilli, Culture Brown C_4, inoculated just below left nipple.

March 4, 1913: Large local lesion. Enlarged hard gland in left axilla. Intradermal tuberculin test + + (positive).

March 24, 1913: Sent to Dr. John F. Anderson, Public Health Service, Washington, D. C. This monkey was ideally infected for an attempt at cure by the Friedmann remedy, but died after a few months.

MONKEY 19.—March 31, 1913: Sixty bacilli, Culture Brown C_4, injected near right nipple.

June 26, 1913: No local lesion or enlarged glands.

Nov. 12, 1913: Subcutaneous tuberculin test (Chart 5) negative.

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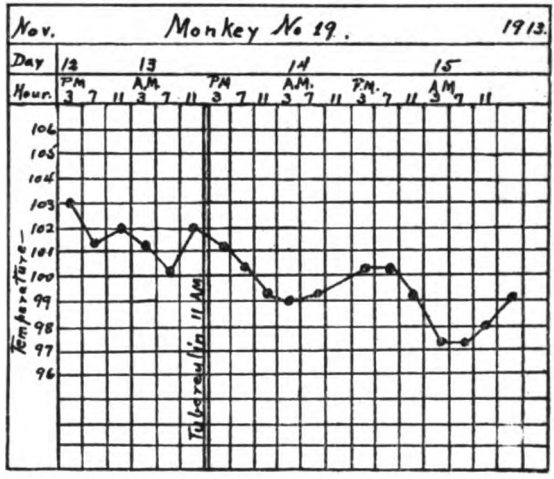

Chart 5.—Temperature chart of Monkey 19.

June 3, 1914: Killed. Necropsy completely negative.

MONKEY 20.—March 31, 1913: Twenty-five bacilli, Culture Brown C_4, inoculated near right nipple.

May 7, 1913: No local lesion.

May 29, 1913: No local lesion or glands enlarged.

Nov. 13, 1913: Tuberculin reaction (subcutaneous test). Notice (see Chart 6) interference with night drop. Probably positive.

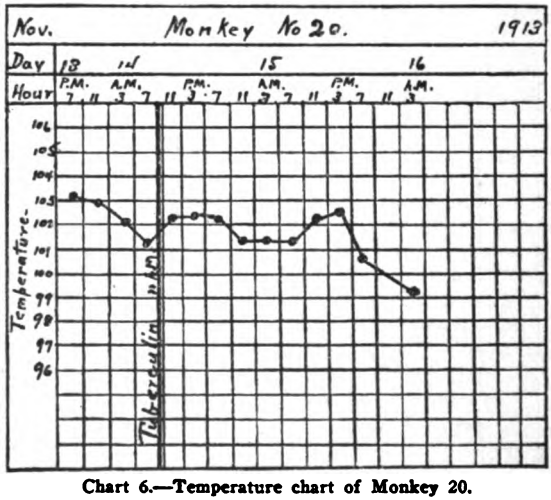

Chart 6.—Temperature chart of Monkey 20.

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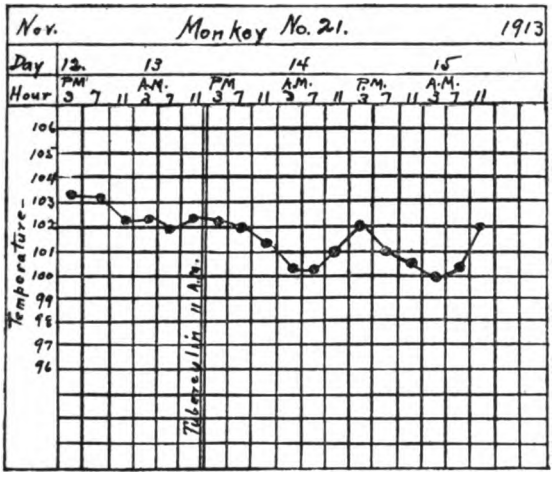

Chart 7.—Temperature chart of Monkey 21.

Dec. 10, 1913: Found dead. Necropsy negative except for a pus-sack in lesser peritoneal cavity. No tubercle bacilli could be found on staining this pus. No enlarged bronchial glands.

MONKEY 21.—Nov. 12, 1913: Subcutaneous tuberculin test (Chart 7) negative.

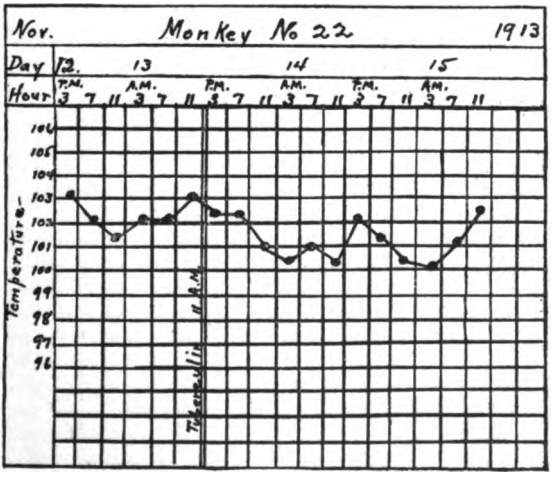

Chart 8.—Temperature chart of Monkey 22.

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Jan. 8, 1914: A burn made above left ankle which produced a linear scar.

Feb. 26, 1914: Three hundred bacilli (Culture Brown C_4) injected into scar. The site of inoculation bled and the blood was reinjected into two other places in scar.

MONKEY 23.—Nov. 12, 1913: Subcutaneous tuberculin test (Chart 9) negative.

Feb. 26, 1914: Three hundred bacilli (Culture Brown C_4) injected into left leg.

Blood Examinations:

MONKEY 21.—Feb. 27, 1914: Platelets 408,000. Whites 12,600. Lymphocyte 64.5 per cent.

MONKEY 23.—Feb. 27, 1914: Platelets 400,000. Whites 11,000. Lymphocyte 54.5 per cent.

MONKEY 21.—March 10, 1914: Platelets 532,000. Whites 16,600. Lymphocyte 64.5 per cent.

MONKEY 23.—March 10, 1914: Platelets 416,000. Whites 17,060. Lymphocyte 57 per cent.

MONKEY 21.—March 12, 1914: Platelets 516,000.

MONKEY 23.—March 12, 1914: Platelets 384,000.

MONKEY 21.—March 25, 1914: Platelets 542,000. Lymphocytes 72 per cent.

MONKEY 23.—March 23, 1914: Platelets 504,000. Lymphocyte 60 per cent.

MONKEY 23.—April 1, 1914: Has a local lump. No enlarged glands.

April 2, 1914: This lump removed under ether.

April 3, 1914: Good Healing. No glands in either monkey.

MONKEY 21.—Platelets 596,000.

MONKEY 23.—Platelets 440,000.

April 28, 1914: Both monkeys killed.

MONKEY 21.—Several tuberculous nodules in liver, also liver abscess. Tuberculous glands in peritoneum. The scar had become ulcerated and had bled easily. No inguinal glands were infected.

MONKEY 23.—Necropsy completely negative.

MONKEY 22.—March 31, 1913: Ten bacilli (Culture Brown C_4) injected just below right nipple.

May 7, 1913: No local lesion.

July 29, 1913: No local lesion or enlarged gland.

Nov. 12, 1913: Subcutaneous tuberculin test (Chart 8) negative.

Aug. 3, 1914: Killed. Necropsy negative.

MONKEY 24.—March 31, 1913: One hundred bacilli (Culture Brown C_4) injected near right nipple.

May 28, 1913: Local lesion, hard lump. Enlarged gland in right axilla.

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May 29, 1913: Under ether local lesion and enlarged gland and axillary gland completely removed.

June 26, 1913: Complete healing, no evidence of enlarged glands or return of lesion.

Sept. 10, 1913: Killed. Portions of left eye and left side of neck swollen. Extensive tuberculosis of lungs, spleen and bronchial glands. Glands over sternum and in neck all purulent. Few tubercles in liver. No local glands in right axilla.

GUINEA-PIG 55.—Dec. 29, 1913: Given 200 bacilli (Lil culture) at nipple area.

Jan. 18, 1914: Local lesion and enlarged inguinal gland.

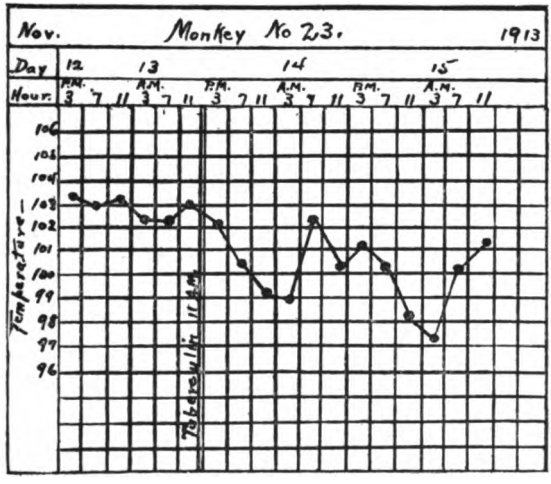

Chart 9.—Temperature chart of Monkey 23.

Jan. 20, 1914: Nipple, local lesion and inguinal glands removed and entire groin cleaned out down to muscles under ether.

March 5, 1914: Groin entirely healed.

April 11, 1914: Killed. Extensive tuberculosis of liver, spleen and lungs.

The following attempt to treat tuberculous guinea-pigs with inoculations of living tubercle bacilli is of interest:

Dec. 19, 1912: Guinea-Pigs 13, 15, 16 and 17, weighing about 500 gm., were each given 25 bacilli of Culture Brown C_4 in right nipple area.

Jan. 13, 1913: Each pig has slightly enlarged and hard right inguinal gland. Pigs 16 and 17 were used as controls. Pigs 13 and 15 were given inoculations of live tubercle bacilli from the same culture as that producing the infection. These

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inoculations were given in the left nipple area, the numbers given being slowly increased from 1 to 60 during a period of twelve weeks.[2]

Table 3 gives the numbers given and the dates.

TABLE 3.—INOCULATIONS INTO GUINEA-PIGS 15 AND 13

Date	Tubercle Bacilli Guinea-Pig 15	Tubercle Bacilli Guinea-Pig 13	Date	Tubercle Bacilli Guinea-Pig 15	Tubercle Bacilli Guinea-Pig 13
1/ 9/13	2	..	2/10/13	20	14
1/13/13	4	1	2/13/13	25	16
1/16/13	6	3	2/17/13	30	20
1/20/13	8	5	2/20/13	35	25
1/23/13	9	6	2/24/13	40	30
1/27/13	10	7	3/ 3/13	45	35
1/30/13	12	8	3/10/13	50	40
2/ 3/13	14	10	3/27/13	50	40
2/ 6/13	16	12	4/ 3/13	60	50

April 21, 1913: Guinea-Pig 13 had discharging sinus in right nipple area. Killed. Large inguinal glands on right side. Large local lesion. Few tubercles in spleen. Otherwise negative.

May 8, 1913: Guinea-Pigs 15 and 16 were killed. Each had dry ulcer and large local lesion. Right inguinal glands were of equal size. Guinea-pig 16 showed no other tuberculous lesions. Guinea-Pig 15 had a few tubercles in spleen and very minute enlarged bronchial glands.

Nov. 22, 1913: Guinea-Pig 17 killed. Local lesion and right inguinal glands same as others. No other tuberculous lesions. This experiment certainly failed to show any favorable action due to the treatment given. Two pigs in the same series, Guinea-Pigs 14 and 18, treated with tuberculin showed much more extensive and widespread tuberculosis.

It has probably been taken for granted that virulent cultures are all of equal potency. By means of the Barber technic of isolating definite small numbers it may be shown that this is not the case.

Table 4 gives a list of certain cultures and the smallest number so far found capable of producing progressive and fatal tuberculosis in adult guinea-pigs.

2. These experiments also prove that a tuberculous guinea-pig is immune to subsequent small lethal doses inoculated subcutaneously. "Römer, and following him Tomarkin, were able to show a sustained resistance of moderately tuberculous guinea-pigs toward a fresh cutaneous inoculation. A similar immunity toward a subcutaneous dose, so far as I know, has not yet been demonstrated."—Smith, Theobald: An Attempt to Interpret Present-Day Uses of Vaccines, The Journal A. M. A., May 24, 1913, p. 1591.

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TABLE 4.—POTENCY OF FATAL CULTURES

Cultures	Minimum Lethal Number
1. B	120 bacilli
2. Barber	35 bacilli
3. Brown C_4	10 bacilli
4. Lil	35 bacilli
5. Bd	10 bacilli
6. Ht	50 bacilli
7. Rgls	10 bacilli

Culture 1 has already been described.[3] Three years later this culture from which 120 bacilli had infected a pig, had so decreased in virulence that 100,000 failed repeatedly to infect a guinea-pig.

Culture 2 has also been described.[4]

Culture 3 was isolated in Saranac Lake from the sputum of an advanced consumptive.

Cultures 4, 5, 6 and 7 were grown from the sputum of advanced active cases of pulmonary tuberculosis by means of the antiformin method grown on Dorset's egg-medium and tested within a few months after first obtained.

The following experiment was undertaken to compare the infection produced by the same dosage in two very young guinea-pigs, A and B, a pregnant female pig, C, a nursing female pig, D, and a normal male pig, E, weighing, respectively, 163, 128, 709, 591, and 573 gm.:

Jan. 13, 1914: Each pig received 60 bacilli, Culture "Lil."
March 29, 1914: A died of extensive tuberculosis.
April 12, 1914: B died of extensive tuberculosis. C, D and E in good health.
June 8, 1914: C, D and E killed. Necropsies showed: C, very extensive tuberculosis; D, no evidence of tuberculosis; E, moderate amount of tuberculosis.

It is of course possible that D failed to receive the bacilli at the time of inoculation. The disease made more rapid progress in the pregnant female than in the normal male and was of a galloping type in the tiny pigs.

3. Webb and Williams: Immunity Production by Inoculation of Increasing Numbers of Bacteria Beginning with One Living Organism, Tr. Sixth International Congress on Tuberculosis, 1908.

4. Webb, G. B., and Williams, W. W.: Immunity in Tuberculosis: Its Production in Monkeys and Children, The Journal A. M. A., Oct. 28, 1911, p. 1431.

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Very young guinea-pigs would seem to be much more susceptible to infection by a definite number of bacilli than the older pigs used.

The following experiments on calves are of interest as they indicate the small number of virulent bovine bacilli which may cause infection, and also that a calf can apparently overcome a mild infection.

Table 5 shows the approximate number of virulent bovine bacilli given to the different calves on the various dates.

TABLE 5.—INOCULATIONS INTO CALVES

Calf No.	Age, Weeks	9/20/11	10/4/11	11/15/11	12/1/11	12/11/11	1/20/12	2/13/12
1.	3	50	...	...	5,000	100,000	1,000,000	50 million
2.	13	50	250	500	5,000	.....	500,000	5 million
3.	3	50	250	500	5,000	100,000	1,000,000	50 million
4.	2	..	...	...	500	100,000	1,000,000	50 million
5.	3	..	...	...	500	5,000	500,000	5 million
6.	1	..	...	...	500	5,000	500,000	5 million
7.	3	..	...	...	.....	.....	.....	1,000
8.	3	..	...	...	.....	.....	.....	1,000

March 13, 1912: The intradermal test of Romer was applied to calves 1-6; 50 mg. O. T. was used for each calf. Calves 1, 2, 4, 5 and 6 were positive.

March 21, 1912: The same test was repeated, and all calves, including Calves 7 and 8, were positive.

April 10, 1912: Given subcutaneous tuberculin test and all temperatures taken for thirty-six hours. Calves 1, 7 and 8 were considered suspicious.

April 12, 1912: Calves 5 and 6 killed. Necropsy: One left internal iliac gland in each contained hard calcified healed tubercles. All other glands normal. Otherwise negative.

Oct. 1, 1912: Subcutaneous tuberculin test repeated. Calf 1, typical reaction. Calf 3, not typical reaction but is sick clinically.

Oct. 8, 1912: Calves 1 and 3 killed. Necropsy: Calf 1, fat and in fine condition. No tuberculosis found anywhere. Calf 3, thin and small. One large gland filled with pus on outer side of each thigh at site of inoculations. Pus contained tubercle bacilli. Other glands and rest of body entirely negative.

June 15, 1914: Calves 2, 4, 7 and 8 have been since tested once with the subcutaneous, and three times with the intradermal method, during the last two years and all tests have been negative.

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Inasmuch as 1,000 bacilli of this culture were able to produce infection in calves 3 weeks old, the amounts given were entirely too large and the increase in dosage far too rapid. Only one calf, Calf 3, had clinical tuberculosis, calcified tubercles were found in two, and still another gave no microscopic evidences of tuberculosis.

The fact that calves 7 and 8 apparently overcame completely the mild infection produced is of unusual interest.

The Barber technic has been of great service in studying the effect of subcutaneous inoculation of minute doses of tubercle bacilli. In the infection of human beings by Nature it has been thought that the bacilli can pass through mucous membranes without the production of a local lesion. In the usual laboratory studies a local lesion following subcutaneous inoculation has been regarded as a sine qua non, and an infection of neighboring glands has been predicated.

In a series of guinea-pigs inoculated with minute (from 10 to 60 tubercle bacilli) infective doses subcutaneously, we have frequently observed the absence of a local lesion, but practically never have failed to find the local glands infected as the following specify:

1. Glands infected but no local lesion, fifty-five guinea-pigs.
2. Glands infected and local lesions, twenty-five guinea-pigs.
3. No glands infected or local lesion, but splenic infection, two guinea-pigs. (These, however, had platelets added to the bacilli.)
4. Failed to get infected, five guinea-pigs.

Our experience with both the intradermal and subcutaneous tuberculin diagnostic tests in monkeys has been rather uncertain and decidedly unsatisfactory. Monkey 6 gave repeated negative results to varying amounts of O. T. given intradermally, and then died with extensive, scattered tuberculosis.

Monkeys 8, 10 and 11 gave positive reactions to 20 mg. O. T. given intradermally, but complete

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necropsies failed to show any tuberculosis. No rise of temperature after the subcutaneous inoculation of tuberculin has been observed. Interference with the normal night drop has occurred rather frequently, as in Monkeys 9 and 12 when the necropsies were completely negative and in Monkey 20, when a small amount of tuberculosis was found at necropsy. Certainly repeated tests would seem necessary to decide whether or not a monkey had been infected.

In our report[4] on immunity in tuberculosis in 1911, mention was made of two children inoculated with Culture B.

The children to-day (June 11, 1914), are negative to the von Pirquet reaction, using 100 per cent. O. T. Five other children were later reported on, but Culture B, which was employed, was found to have become a virulent, 200,000 bacilli of this culture failing to infect guinea-pigs.

One of these children (L), however, was so badly exposed, living in a three-room house with an inebriate consumptive in the last stages, that an effort was made to immunize if possible with a more virulent culture. At that time the graded inoculation of this culture (Brown C_4) had not infected a guinea-pig. The child was inoculated beginning Jan. 20, 1913, at seven-day intervals with 2, 3, 5, 7, 10 and 12 bacilli, respectively. Immediately previous to the inoculations a von Pirquet test was doubtful or negative.

March 1, 1913: A slight nodule observed on right forearm at site of a former inoculation. This nodule was at first mistaken for a staphylococcus infection.

March 10, 1913: Foregoing nodule larger. A second smaller nodule 2 inches distant, and a very minute nodule on left forearm.

March 11, 1913: These three nodules removed under chloroform anesthesia. The section of larger nodule showed numerous bacilli.

March 18, 1913: A gland noticed in right axilla enlarged. Removed.

June 14, 1914: Temperature taken through four months remained constantly normal. Child in perfect health. No evidence of glandular enlargement anywhere. Platelets, 396,000. Lymphocytes, 50 per cent. Von Pirquet tuberculin test positive.

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BLOOD-PLATELETS AND TUBERCULOSIS

It has for a long time been observed that blood-platelets are increased in tuberculous infection. Osler, however, has written a scathing rebuke to the tyro who every little while claims to recognize tuberculous infection by increase of these blood elements. It seemed wise to try and investigate why Nature increases these so-called third corpuscles, knowing how rarely if ever she operates without a purpose. We have published elsewhere[5] the following results of our observations.

1. Blood-platelets are increased in the tuberculosis of man and guinea-pigs.

2. Blood-platelets added to a small infective dose of virulent tubercle bacilli appear to modify the infection when injected into guinea-pigs.

In addition we have observed that blood-platelets are increased 12 per cent. in the blood of man and guinea-pigs at an altitude of 6,000 feet. They are also increased by hyperemia of the marrow of long bones and in poisoning by carbon monoxid.

The increase of blood-platelets in the infected Monkey 21 occurred two weeks after the inoculation of virulent tubercle bacilli, and was maintained. The number in the control animal, Monkey 23, remained consistently normal, and post-mortem examinations gave evidence that the findings had been correct.

CONCLUSIONS

1. Guinea-pigs can probably not be immunized with virulent human tubercle bacilli of cultures from which ten bacilli can cause infection.

2. The minimum lethal dose of different virulent cultures would appear to vary in number.

3. The minimum lethal dose for a *Macacus rhesus* is possibly a little larger than that for a guinea-pig.

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5. Webb, Gilbert and Havens: Colorado Med., January, 1914; Trans. Tenth Annual Meeting Nat. Assn. for Study and Prevention of Tuberculosis.

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4. It is necessary to be guided by the virulence of human tubercle bacilli as tested in the guinea-pig before the application to children.

5. After four years two children each inoculated up to five times the lethal dose for a guinea-pig are still negative to the von Pirquet reaction.

6. In monkeys the decrease of lymphocytes as indicated in differential blood-counts would appear to be more reliable in indicating tubercle infection than the tuberculin tests.

7. Less than ten virulent human tubercle bacilli can infect a child.

ABSTRACT OF DISCUSSION

Dr. William Ophüls, San Francisco: What were the anatomic findings in the monkeys that were so infected? Using these very small doses of tubercle bacilli for the infection, one approximates, I presume, the natural infection more closely.

Dr. G. B. Webb, Colorado Springs, Colo.: No observations were made on the tuberculous monkeys except as stated in my paper. We usually killed the monkeys immediately infection was recognized, as indicated either by a local lesion or glandular swelling.

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THE HECHT-WEINBERG REACTION AS A CONTROL OVER THE WASSERMANN REACTION

A STUDY BASED ON ONE THOUSAND PARALLEL TESTS WITH BOTH METHODS

R. B. H. GRADWOHL, M.D.
ST. LOUIS

The massed statistics of Wassermann workers have shown clearly that this reaction, following the classic technic of Wassermann, using the most exact methods of titration of all the reagents, working with the most sensitive antigens whether cholesterinized or not, is *not* uniformly present in all cases of manifest syphilis from a clinical point of view. While we have proved its undefinable value as a diagnostic symptom in many cases of occult syphilis, still we must admit that at times it is consistently negative in many syphilitic conditions. There are times, too, when the reaction disappears entirely independent of treatment. At such times, the serologist is unable to assist the clinician in his efforts to arrive at a correct diagnosis or to check treatment. The disrepute into which the classic Wassermann has at times fallen in the estimation of some practitioners is not so much from the errors that have been so frequently made by incompetent serologists as it is from the *negativity* of the reaction in cases that display unquestionably syphilitic aspects, either cutaneous or visceral.

We have, therefore, been compelled to look to some other test to use, either as a substitute for the Wassermann, or, at least, as a sensitive control of the reaction in cases in which the Wassermann is absent. One of the main objections that has been raised against

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the test in some quarters is the fact that the straight Wassermann technic embodies the use of an amboceptor or hemolytic serum made by injecting sheep's blood into rabbits and using sheep's corpuscles as the basic material around which the remainder of the reaction is made to revolve. This objection has been well taken; for we know from actual investigation that nearly every individual carries in his blood-serum some quantity of antisheep amboceptor resultant from the ingestion of mutton. The number of people who have natural antisheep amboceptor is large. In this country my own figures show, from routine examinations, that 98 per cent. of the American people harbor greater or less quantities of this substance. Granting that this is true, we are forced to admit that a Wassermann reaction carried out in the case of an individual with a high amount of natural antisheep amboceptor and a low amount of syphilitic antibodies will usually result negatively even though the individual is syphilitic.

My attention was first called to this fact some months ago by the admirable work of Leredde and Rubenstein in Paris, who convinced me by a review of their data that the failure to obtain a positive Wassermann reaction in many cases was due to the presence of an undue amount of natural antisheep amboceptor in human serums. Leredde and Rubenstein, reporting a parallel series of tests, using both the straight Wassermann technic and a method which utilized the natural amboceptor in unheated human serums (namely, the Hecht-Weinberg test), showed that the latter test developed a positive reaction in 10 per cent. more cases of syphilis than did the Wassermann. Again, it has been argued that the heating of serums to 56 C. (132.8 F.), such as is practiced in the Wassermann technic for the purpose of ridding the serums of natural complement, develops *complementoid* substances whicn naturally give a false

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conclusion. Again, it has been asserted that the heating of serums destroys a part of the syphilitic antibody on which the reaction depends. It is for these three reasons that Hecht and Weinberg developed a technic that utilized the amboceptor and complement present in practically all blood-serums and refrained from heating the serums by using "active" or unheated serums. The method of Margherita Stern is designed to utilize the natural complement of blood-serums but pays no attention to the natural amboceptor—a serious drawback to the most accurate information for the clinician.

The observations of Hecht and Weinberg, Leredde and Rubenstein and Levaditi convinced me of the advisability of following out the technic of Hecht and Weinberg for purposes of control. Leredde's figures are based on a parallel series of blood-tests in 1,636 serums. My own investigations are confined to 1,000 parallel observations. The technic of Hecht-Weinberg, which I have worked out, is the following:

Place in a rack fourteen small test-tubes. The first ten of these tubes are used to determine the hemolytic index of the suspected blood. By this we mean the exact amount of hemolytic amboceptor present in the given blood-serum. The last four tubes are used in the actual test. Add 0.1 c.c. of fresh unheated patient's blood-serum to each of the first ten tubes. Then add decreasing amounts of normal salt solution to these tubes, beginning with 1 c.c., then 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 c.c. to the succeeding nine tubes. Next, add increasing amounts of fresh 5 per cent. suspension of sheep's blood starting with 0.1 c.c. and ending with 1 c.c. Place the rack in the water-bath for one-half hour. The tube which last shows complete hemolysis constitutes our "hemolytic index;" if it is Tube 4 our index is 4, because this tube had received 0.4 c.c. of sheep's corpuscles. The index determines the amount of sheep's corpuscles to

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be added to the last four tubes. The first three tubes (11, 12 and 13) constitute the tubes for the actual test, while the last tube in the rack (Tube 14) serves as our serum control tube. Tubes 11, 12 and 13 receive, therefore, the patient's serum, the proper amount of sheep's corpuscles (dependent on the hemolytic index) rising strengths of antigen, but no complement and no amboceptor. Tube 14 receives only sheep's corpuscles but no antigen. In our technic we use 0.1 c.c. of a diluted antigen determined by titration in Tube 11, 0.15 c.c. antigen in Tube 12 and 0.2 c.c. in Tube 13. In order to equalize the volume of fluid in all these tubes, we add 0.2 c.c. normal saline to Tube 11, 0.15 c.c. to Tube 12 and 0.1 c.c. to Tube 13, and 0.3 c.c. to Tube 14. The tubes are then agitated and placed in the water-bath for half an hour. These last four tubes are filled at the time we make the additions to the first ten and are left with them in the water-bath for one-half hour for fixation of complement, the rack is then taken out and the hemolytic index computed. If the index is low, say from 1 to 4, we add 0.1 c.c. of sheep's blood to the last four tubes. If the index is between 5 and 7 we use 0.15 c.c. sheep's blood and if it is between 7 and 10 we use 0.2 c.c. In our experience in this country we have never found an index above 10, although in France it is not uncommon to obtain an index of 15 or 17.

If the patient's serum has an index below 3, we regard the reaction as of doubtful value. If it is above 3, we regard it as absolute. The reaction is read off exactly as is the Wassermann, that is, inhibition or non-inhibition of hemolysis.

This method utilizes the natural complement present in nearly all human blood and the natural amboceptor present in at least 98 per cent. of blood-serums. If the amount of complement or natural antisheep amboceptor is very low, we can add the proper amount of

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guinea-pig's serum or rabbit's immune serum, ascertained by preliminary titration. The particular advantage of this test is the fact that we are dealing with unheated serums, avoiding the chance of the development of complementoid substances. I wish to state, in passing, that the Hecht-Weinberg test has never been found negative in any blood-serum which showed a positive Wassermann, but it has frequently been found positive in blood-serums that show a negative Wassermann. Thus, its advantage can readily be seen. We do not offer these conclusions for the purpose of eliminating the Wassermann, but we do most candidly confess that provided there is sufficient natural amboceptor present, it has superior advantages to the Wassermann. Our practice is to perform both tests, one serving as a check on the other.

The group of cases in which the amount of natural amboceptor in human serums seems to play a definite rôle in making a Wassermann appear negative and a Hecht-Weinberg positive, comprise the following:

1. Cases of ocular syphilis.

2. Cases of visceral syphilis, particularly of the liver and heart.

3. Cases of syphilis that have received intensive but inadequate treatment.

4. Provocative cases in which we are seeking to revive a Wassermann in suspected cases.

5. Cases of monosymptomatic tertiaries.

In general, in cases of what might be termed "limited" syphilis, this modification will be found of great use. With Leredde and Rubenstein, Latapie, and Hecht and Weinberg, I believe that the use of the Hecht-Weinberg test as a control over the Wassermann will bring to light many cases of occult syphilis about which the clinical diagnosis is often uncertain. Our rule is to do both tests, and as a result of this practice *we can offer a series of one thousand cases in*

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which the Hecht-Weinberg was positive 15 per cent. more often than the Wassermann. We regard this "dissociated reaction" as diagnostic of syphilis. The series studied represents every phase of true syphilis from the primary stage through secondary, tertiary and so-called metasyphilitic conditions. We regard the Hecht-Weinberg test as the best modification that has come forward to serve as a check on the classic method of Wassermann, Neisser and Bruck.

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THE BLOOD IN ACIDOSIS FROM THE QUANTITATIVE POINT OF VIEW

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Although the elimination of acetone bodies, found in the urine in various conditions, has been thoroughly investigated, we still lack reliable information as to the extent to which these substances may accumulate in human blood and tissues during life. Diminished alkalinity of the blood, such as may occur in acidosis, is a condition unfavorable to diuresis, so that it is quite possible to have only a moderately high excretion of acetone bodies in the urine of a patient who may exhibit symptoms of severe acid intoxication. It is in such cases that an analysis of the blood should yield data of diagnostic and prognostic value.

By a method, which is described elsewhere,[1] it is possible to determine acetone, aceto-acetic acid and beta-oxybutyric acid in small amounts of blood drawn directly from a vein. From 2 c.c. to 5 c.c. of blood is sufficient, and the analysis may be carried out in about the same time as is required for an analysis of the urine for the same constituents. When analyzed according to this method, normal blood from man, dog, pig and beef is found to contain less than 1.5 mg. of gross aceto-acetic acid (acetone preformed, plus aceto-acetic acid), and less than 4 mg. of oxybutyric acid per 100 gm. of blood, all results being expressed in terms of acetone.

A number of pathologic conditions associated with acidosis have been studied, including light and severe diabetes, febrile conditions of childhood, postoperative

1. Marriott: Jour. Biol. Chem., August, 1914.

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acidosis and experimental phloridzin and pancreatic diabetes in dogs. In all these conditions the acetone bodies were present in the blood in excess of the normal. The highest figures obtained were from the blood of a patient with severe diabetes at a time when he exhibited signs of impending coma. In this instance, the oxybutyric acid amounted to 45.2 mg. per 100 gm. and the gross aceto-acetic acid to 28.0 mg., both calculated in terms of acetone.

The accumulation of the acetone substances in the blood and the excretion of these substances in the urine did not always run parallel. Administration of alkali resulted in considerable alteration in the relative distribution of the acetone substances between the blood and the urine, the effect being to diminish very considerably the concentration of these substances in the blood, without at the same time bringing about any appreciable change in the total daily output in the urine.

Analyses of the blood for preformed acetone indicated that this substance may amount to more than 60 per cent. of the gross aceto-acetic acid, in conditions of severe diabetes. With alkali therapy, preformed acetone showed a relative diminution. That is to say, the alkali appeared to decrease the decomposition of aceto-acetic acid.

In the bloods examined the ratio of oxybutyric acid to total acetone substances ("beta ratio" or Kennaway, Pembrey and Poulton) varied from 50 per cent. to 80 per cent. The significance of this ratio is not, as yet, entirely apparent. It is possible that a high ratio may be an indication of partial asphyxia; for we know from the work of Embden, Friedmann, and Dakin and their collaborators that, with diminished oxygen supply, various organs of the body readily effect the reduction of aceto-acetic acid to oxybutyric acid, whereas the reverse change is favored by the presence of an abundance of oxygen.

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So far I have spoken only of the accumulation of the acetone substances in the blood, and have said nothing of the tissues. The blood, however, appears to be a fairly satisfactory index of the extent to which acetone substances may accumulate in the tissues. For example, it was found that the blood and liver of a starved phloridzinized dog contained the same amounts of acetone bodies per 100 gm. and the muscles approximately one-half as much. The beta ratio, however, was higher in the muscles and liver than in the blood, and this would, of course, be expected as there would be relatively less oxygen in these organs than in blood.

It is not intended here to present a detailed discussion of acidosis but merely to indicate some applications of a method which I believe to be capable of throwing light on the subject of acetone body acidosis.

Washington University Medical School—Laboratory of Biological Chemistry.

ABSTRACT OF DISCUSSION

Prof. A. J. Carlson, Chicago: I should like to ask Dr. Marriott for the findings in the pancreatic diabetes in dogs. It has been my experience in this experimental diabetes that there is not in the urine or in the breath of these dogs the characteristic acetone odor that is found in advanced cases of human diabetes. Was there any indication of increase of acetone in the experimental dogs comparable to that in cases of human diabetes?

Dr. W. McKim Marriott, St. Louis: I was surprised to find that in pancreatic diabetes in the dog there was only the very slightest increase in acetone bodies, nothing at all comparable to that of human diabetes or even of phloridzin diabetes in the dog; the figures were just above the normal.

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PARENTERAL PROTEIN DIGESTION

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INTRODUCTION

The units of life in our bodies are the cells of which our organs and tissues are composed. In order that we may be in health, all the cells of our bodies must be in normal condition and must function in harmony. Inasmuch as these cells are living, growing and multiplying, they must have a supply of normal food, and since these cells are made up of protein to a large extent they must be supplied with protein food. The digestive functions of the alimentary canal have been developed for the purpose of preparing proteins for the body-cells to feed on. The proteins of our food are normally broken up in the digestive tract into amino-acids. These are absorbed through the intestinal walls and serve for the nourishment of the body-cells. The blood and lymph carry to all the cells of the body the prepared and absorbed protein split products. Each kind of cell selects from these products such portions as it needs in its own metabolism. It sometimes happens that proteins reach the blood and lymph without being acted on by the digestive juices of the alimentary canal. On a small scale this is constantly happening. We absorb minute quantities of protein through the mucous membrane of the respiratory tract, and in ordinary health it may happen that small amounts of undigested or imperfectly digested proteins are absorbed through the intestinal wall. In the therapeutic employment of serums and vaccines undigested protein, sometimes in large amount, is introduced into

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the circulation. In case of infection, living proteins find their way into the body and there grow and multiply, converting the simple soluble proteins of the body-fluids into complex, foreign proteins. When foreign proteins find their way into the body by any of these methods they must be digested by the agency of ferments supplied by the body-cells. This process we designate as "parenteral digestion" in contradistinction to the normal "enteral digestion" which goes on in the alimentary canal. Up to the present we know but little concerning the action of the parenteral proteolytic ferments or their digestive products. We know that at one stage in enteral digestion the protein molecule yields an active poison, but in normal digestion this is split into harmless bodies before absorption. In parenteral digestion this poison is set free in the blood and tissues and gives rise to the symptoms of the infectious diseases.

How to control and modify parenteral protein digestion is now one of the big problems in experimental medicine.

The body-cells feed on the pabulum brought to them by the blood and lymph. The wandering cells, such as the leukocytes, may go in search of food, and observation has shown that they are attracted to localities in which there is accumulation of protein material, as to the intestinal walls during the process of digestion, to local infections and to points where non-diffusible proteins, living or dead, are injected. But most cells of the animal body are fixed and can act only on pabulum brought to them. The blood and lymph carry in solution both proteins and protein split products, the latter as amino-acids. Of these split products each kind of cell selects such as it may require in its metabolic process. To what extent body-cells are nourished by the amino-acids absorbed as such from the alimentary canal we do not know. Indeed, until recently it was believed that all the amino-acids formed in enteral digestion are synthesized into albumins and globulins

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either during absorption or soon thereafter. That this is the fate of the greater part of these protein split products is certainly true. The diverse proteins of the food are split into amino-acids in the intestine and synthesized into specific proteins, peculiar to the species, and these are distributed through all the tissues by the blood and lymph and constitute the protein food-supply of the cells. Therefore, the bulk of the protein food carried to the various organs is the same and each kind of cell must split this in such a way that the cleavage products can be utilized in its own particular structure. The liver-cell must split the albumins and globulins, one or both, in such a way as to serve its own needs, and the pancreatic cells must obtain from the same raw material, building stones cut to fit its peculiar masonry. The agencies with which this work is accomplished are known as ferments. We do not know the exact nature of any ferment. We can recognize their presence only by their effects. There must be as many kinds of ferments as there are kinds of cells, and each ferment is specific and cleaves the substrate along its own peculiar lines. From this it must follow that the cell peculiar to each organ in the animal body supplies its own specific ferment or ferments and it is by virtue of this that the cell continues to live. This conclusion reached by reasoning has been demonstrated by experiments.

DIGESTION IN UNICELLULAR ORGANISMS

Experimental studies of digestive processes in unicellular and other low organisms have been numerous and the literature thus developed is too voluminous to be reviewed in detail. I will content myself with notice of some of the more important contributions such as enable us to understand cellular digestion in general. Leidy[1] made many observations on digestion in amebas. In the first place he called attention to the fact that amebas feed on algae and that they are most

1. Leidy: Fresh Water Rhizopods of North America.

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numerous where their food-supply is most abundant. He watched the ingestion of the food which is accomplished by two pseudopodia which enclose the infusorian which now lies in the vacuole and gradually loses all structure and ultimately disappears. Large amebas (*A. proteus*) feed on smaller species (*A. verrucosa*). Engulfed algae gradually turn brown while being digested. Kölliker[2] made similar observations and found the average time between ingestion and the extrusion of the indigestible portion to vary from two to six hours. Carter[3] points out that the ingestion, digestion and egestion of food substances distinguish amebas from plant-cells. Haeckel[4] regards the intracellular digestion in rhizopods as a chemical process, but fails to make any satisfactory demonstration of this belief. Greenwood,[5] from his studies of digestion in amebas, comes to the following conclusions: 1. Starch is ingested on contact but is not digested. It may be retained as long as six days, but is then rejected without change. 2. In regard to fat he is left somewhat in doubt. Milk globules are included, but after twenty-four hours they give normal and ready reaction with osmic acid. The ultimate disposal of fat was not satisfactorily determined. 3. Naked proteins (monads) are quickly and thoroughly digested by amebas. 4. Shielded proteins (enclosed by cellulose walls, such as *Torulae*) are slowly digested without break in the cellulose wall. The last point is important. It shows that the ameba elaborates a digestive fluid which penetrates the cellulose wall and digests the contents.

Next come the highly important experiments of le Dantec,[6] which show that in amebas and all infusoria, so far as tested, intracellular digestion is accompanied by the development of acid. The fluid of the vacuole

2. Kölliker: Ztschr. f. wissensch. Zool., 1849.
3. Carter: Ann. Nat. Hist., 1863.
4. Haeckel: Ztschr. f. wissensch. Zool., 1865.
5. Greenwood: Jour. Physiol., vii, 253.
6. Le Dantec: Ann. de l'Inst. Pasteur, 1890, 1891.

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surrounding the captured and ingested food becomes acid. The nature of this acid has not been positively determined, but there is no proof that it is not hydrochloric acid, and the weight of experimental evidence is in favor of its being this mineral acid. It must be understood, however, that as in the gastric juice, the acid is not the sole and effective agent. Any substance ingested by amebas, without regard to its nutritive character, starts the secretion of the acid, but only nutritious substances are digested. It follows, therefore, that we must assume the existence of digestive ferments which are active in acid fluids as is the case with the pepsin of the gastric juice. It should be stated that Krukenberg[7] found that the glycerin extract of the cellular substance of certain unicellular organisms contains a proteolytic ferment which is active only in the presence of acid.

There is no animal from the lowest to the highest, so far as investigation has been made, which does not elaborate one or more proteolytic ferments. The only organisms in which the truth of this assertion might be questioned on *a priori* grounds are parasites, which might be supposed to depend on their hosts for predigested food. Indeed, some of the first studies made on digestive processes in certain intestinal worms failed to detect the presence of specific ferments, but more thorough investigations have shown that intestinal worms elaborate proteolytic ferments which serve their own metabolism and antiproteolytic bodies which protect them against the digestive fluids of their hosts. The universally distributed proteolytic ferments of the animal kingdom are divided into those which are active in acid, and those active in alkaline solutions. The former are generally designated as pepsins and the latter as trypsins.

In unicellular organisms the chief digestive processes are intracellular. Indeed, with many of these

7. Krukenberg: Kuhne's Untersuch., 1882, ii.

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low forms of life it has not been possible to demonstrate extracellular digestion; we are not justified, however, in denying its existence. The yeast-cell secretes a diastatic ferment which rapidly acts on sugar, but its proteolytic ferment is, under normal conditions, retained within the cell in which it functions. Since no known animal can live without protein food and since every species contains its own specific proteins, each living animal must produce proteolytic ferments. As the structure of the animal body becomes complicated and differentiation takes place, certain cells are set apart to do the preliminary digestion for the whole, but each cell must digest its own special food. Therefore, Weinland[8] is right when he states that intracellular digestion occurs in all animals.

DIGESTION IN BACTERIA

It has been generally assumed that bacteria are low forms of plant life, but researches in my own laboratory, confirmed by later studies in Kossel's laboratory, show that bacteria contain no cellulose and are particulate, unshielded proteins and consequently are more nearly related to low forms of animal life. I should not classify bacteria as either plants or animals. In their life processes they are more closely related to animal life. Some bacteria, possibly all, can utilize split proteins in their metabolism, but under ordinary conditions they feed on unbroken, but relatively simple proteins. All bacteria, so far as studied, produce specific proteolytic ferments. In some, only intracellular ferments have been detected; in others only extracellular; in still others both are in evidence. The liquefying bacteria pour out proteases which diffuse widely through the medium and visibly demonstrate their activity. The abundance with which a given bacterium elaborates its protease is subject to many conditions. As a rule, the richer the medium in carbohy-

8. Weinland: Oppenheimer's Handbuch der Biochemie.

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drates, the less abundant is the secretion of protease. It is probable that the presence of certain proteins in the medium stimulate the flow of the digestive agent, though, so far as I know, this has not been experimentally demonstrated. From analogy with other living cells, this is the natural assumption. For the most part bacterial proteases act in alkaline solution and, therefore, are classified as trypsins. This, however, is not without exception. Many bacteria which grow in milk and milk products digest proteins in acid mediums.

There are two points about the action of bacterial proteases which I feel justified in emphasizing. One is the fact that their activity is delayed by quinin, antipyrin and other antifebrile medicaments. This may in part at least explain the action of these drugs. The second is the fact that so far as experimental evidence has gone, no bacterial protease is capable of digesting living protein. This seems to me of great importance. The prevailing idea among morphologic pathologists seems to be that the lesions of the infectious diseases are due to the digestive action of the bacterium on the body-cell. The typhoid bacillus is supposed to feed on the walls of the intestine and cause ulceration, and the spirochetes are believed to induce the lesions of tertiary syphilis in like manner. Everything known up to the present time concerning the digestive action of bacteria is contradictory to these views. It seems quite certain that during the period of incubation of an infectious disease, the invading organism supplies the ferment, the simple, soluble non-organized proteins of the body of the host constitute the substrate, the process is largely synthetic and constructive, leading to the conversion of the simple proteins of the host into the more complex, cellular proteins of the invading organism, no marked protein cleavage occurs, no poison is set free and consequently there is no recognizable disturbance in the health of

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the host. On the other hand, during the active stage of an infectious disease the cells of the host supply the ferment, the complex, cellular proteins of the invading organism constitute the substrate, the process is essentially analytic and destructive, and the protein poison is set free and causes the symptoms and lesions of the disease.

It should be stated that the synthetic action of bacterial proteases, such as I have spoken of as occurring in the growth and multiplication of bacteria, whether in the test-tube or in the body, is not an assumption, but a demonstrated fact. The peptone of the culture-medium is built into bacterial cellular protein. Zak[9] has shown that pyocyaneus protease *in vitro* synthesizes simple proteins into more complex ones.

LEUKOCYTIC DIGESTION

The phagocytic theory of Metchnikoff and the work which that distinguished investigator has done in its support are too well known to need statement, and I will confine myself to a brief review of what has been done in the study of leukocytic digestion. Leber[10] showed that sterile pus liquefies gelatin and digests fibrin. Blood-serum plates are now employed in determining the relative digestive action of leukocytes from different sources. A platinum loop carrying a few leukocytes is brought into contact with the plate at several points and the plate is then kept at from 50 to 55 C. (122 to 131 F.) for twenty-four hours. This temperature favors the digestive action of the ferments, and retards or inhibits the growth of bacteria that may be present. Each leukocytic colony digests the serum and forms a lacuna. From the depth of these lacunae the relative digestive activity of each leukocytic colony may be determined. This difference may be demonstrated by comparing the action of leukocytes from myelogenous leukemia with those from

9. Zak: Beitr. z. chem. Physiol. u. Pathol., 1912, x, 287.
10. Leber: Die Entstehung der Entzündung, 1891.

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lymphatic leukemia. The blood from a case of myelogenous leukemia partially digests itself on being kept for seventy hours at 55 C. This autodigestion and the extent to which it occurs may be demonstrated by the presence and amount of albumoses formed as was first shown by Erben.[11] It should be understood, however, that normal leukocytes have a digestive action, as was first demonstrated by Pfeiffer.[12]

The protease of leukocytes may be obtained best from the blood of a case of myelogenous leukemia, by precipitating a properly prepared plasma—leukocyte mixture with alcohol, allowing to stand for some months, and then extracting the precipitate with glycerin. Similar preparations have been made from the spleen, bone-marrow and from sterile abscesses induced by subcutaneous injections of turpentine. The various methods of preparing leukocytic protease are given by Jochmann.[13] It is believed by Jochmann and others that the leukocytic protease is supplied wholly, or at least, most abundantly, from the neutrophil cells. It has been obtained from leukocytes, colostrum corpuscles, the saliva and the lochial secretion. It is supposed to digest excess of milk accumulating in the gland and possibly it may be of service to the child during the first few days of extra-uterine life. That of the lochial discharge may aid in the cleansing of the uterus.

The value of the leukocytic protease in causing resorption from wounds, in cleansing abscesses and in all local infections is well understood. It is well known that tuberculous pus, so long as it is due to an unmixed infection, is quite free from this ferment and it is believed that injections of iodoform in tuberculous, local infections bring to the site polynuclear cells which aid in effecting cure by their secretions. Whenever foreign proteins appear in the body, the leuko-

11. Erben: Wien. klin. Wchnschr., 1902, p. 276.
12. Pfeiffer: Wien. klin. Wchnschr., 1906, No. 42.
13. Jochmann: Kolle and Wassermann's Handbuch, Ed. 2, ii, 1301.

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cytes assemble and endeavor to destroy the undesired material by digesting it. It seems that the ferment is stored up largely in the leukocytes and that the most effective digestive action does not occur until the leukocytic cells disintegrate. In case of local bacterial infection many bacteria are taken into the leukocytes, and in some instances at least intracellular digestion proceeds much in the same way as it does in amebas. It is held by Müller and others that resolution in pneumonia is the result of the activity of the leukocytic protease, which becomes quickly effective from the dissolution of a large number of leukocytes. According to Bittorf[14] the urine after resolution in pneumonia quickly digests fibrin. Abnormal activity of this protease may be followed by the appearance of the digestive products in the form of albumose in the urine. This is the explanation of peptonuria in cases of chronic suppuration. When a large number of leukocytes disintegrate in a confined space the ferment set free digests any fibrin, lymphocytes and red corpuscles that may be present. The autodigestion of leukocytes leads to the production of ammonia, amino-acids and nuclein bases. Tuberculous pus does not undergo autodigestion. According to Jochmann a spleen, as large as a child's head, from a case of myelogenous leukemia, kept at 55 C., will within twenty hours break down into a dark red fluid in which the connective tissue floats. A normal spleen passes through the same change in about twice the time. The liquefied autolysate contains ammonia, leucin and tyrosin in abundance. The bactericidal action of the leukocytic protease has been studied by Nuttall, Buchner, Schattenfroh, Peterson, Kling, myself and others. One of the important questions is that of the identity of the bactericidal and the proteolytic constituents of blood-serum or of leukocytic extract. There is not time to enter the literature of this subject here.

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14. Bittorf: Deutsch. Arch. f. klin. Med., 1913, p. 91.

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It must be evident from what has been said that the leukocytes are constantly supplying the blood and bathing the tissues with an active proteolytic ferment. Were there no regulation of this process the body would digest itself. There is constantly in the blood an antiferment. The relation between these bodies and its regulation are among the most interesting and important matters in experimental medicine. It seems to be a well-established fact that repeated injections of a ferment into an animal leads to the production of antibody. Such bodies have been produced in this way by injections of pancreatic trypsin, pepsin, rennin and fibrin ferment, and it seems justifiable to suppose that the antiferment or antitrypsin of the blood originates in this way. Indeed, Jochmann and Kantorowicz have increased the antitrypsin of blood-serum to sixty-four times the initial amount by repeated injections of leukocytic trypsin. These investigators believe that the antitrypsin of the blood is identical with pancreatic antitrypsin, and that the former may owe its development to increase in either the leukocytic or the pancreatic trypsin. This means, of course, that the two ferments are identical and their antibodies are. There are many and diverse theories concerning the relation between leukocytic trypsin and its antibody. Laboratory methods of measuring the antitrypsin strength of blood-serum and rules are given for the interpretation of the results. Wiens[15] finds a low antitrypsin content in the blood-serum in all infectious diseases in which the polynuclear corpuscles are increased, and he regards an opposite finding as unfavorable in prognosis. Theoretically he holds that the greater the number of the polynuclear cells, the more abundantly will the trypsin be supplied and the greater the number of bacteria destroyed. Other investigators have reached radically different conclusions that is, they find that those cases do best in which the antitrypsin con-

15. Wiens: Deutsch. Arch. f. klin. Med., 1906, p. 91; Centralbl. f. inn. Med., 1907.

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tent is high. It seems to me that a great danger to the life of the infected man lies in the too rapid cleavage of the bacterial proteins, and at one time a rise in the antitrypsin content may save life. The relation between the leukocytic trypsin and its antibody must be a delicately adjusted one and one which fluctuates from hour to hour. Brieger and Trebing[16] and others have found the antitrypsin titer high in cancer, advanced tuberculosis, diabetes and the severe anemias, and they call it a cachexia reaction. Here again it seems most plausible that the large amount of waste protein material in the blood endangers life in case of its rapid digestion, and the large amount of antibody supplied is a protective agent. Evidently the blood is a digestive, as well as nutritive fluid, and the digestive processes taking place are regulated by a finely adjusted mechanism which at present we do not understand.

There has been for some years increasing evidence that the enzyme-inhibiting constituent of blood-serum is a lipoid. So far as I know the literature, this was first suggested by Schwartz,[17] who found that the inhibiting action of serums could be lessened by extraction with ether and restored by the addition of lecithin. Sugimoto[18] found that the extraction of serums with ether and benzol has the same effect. Jobling and Peterson[19] have apparently settled the question and reached the following conclusions: 1. The ferment-inhibiting action of the serum is due to the presence of compounds of the unsaturated fatty acids. 2. These fatty acid compounds may be removed from the serum by means of chloroform or ether. 3. Soaps prepared by saponifying the chloroform or ether extracts inhibit the action of trypsin. 4. The antienzyme action of the serum can be removed by filtering acid serum

16. Brieger and Trebing: Berl. klin. Wchnschr., 1908.
17. Schwartz: Wien. klin. Wchnschr., 1909, xxii, 1151.
18. Sugimoto: Arch. f. exper. Path. u. Pharmakol., 1913, lxxiv, 14.
19. Jobling and Peterson: Jour. Exper. Med., 1914, xix, 459.

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through kaolin, and can in part be restored by extracting the kaolin. 5. The decrease in strength of antienzyme in old serums is probably due to the action of serum lipase. 6. Iodin, potassium iodid or hydrogen peroxid removes the inhibiting action of the serum. 7. Soaps of the unsaturated fatty acids lose their ferment-inhibiting action when heated with serum at 70 C. (158 F.).

Leukocytes elaborate in addition to the protease, a lipase and an oxidase which we pass by since they are not germane to the subject under discussion.

Jacoby[20] and Richet[21] have shown that the liver-cells elaborate a specific proteolytic ferment, and Abderhalden[22] and his students, by the optical method, have demonstrated that: 1. Infusions of liver tissue digest liver peptone, but not peptone prepared from the kidneys or thyroid. 2. Infusions of thyroid digest thyroid peptone, but not peptones prepared from liver or kidney. 3. Infusions of kidney digest both kidney and liver peptones and to some extent that from the thyroid. 4. Infusions from muscle digest muscle peptone but not peptones prepared from liver or brain. 5. Peptone prepared from testicles is digested by infusions from testicle or from kidney. While more work along this line is highly desirable it seems safe to conclude that each kind of cell in the animal body elaborates one or more specific proteolytic ferments and that the conclusions reached by pure deduction are confirmed by experiment.

It is well established that the products of the cleavage of complex proteins by ferments vary within certain limits. A protease may digest certain proteins and have no effect on others. Some unicellular organisms feed only on certain proteins. The phyloxera feeds only on the grape-vine. Some organisms must pass one or more stages in their development as para-

20. Jacoby: Hofmeister's Beitr., 1903, iii, 446.
21. Richet: Compt. rend., Soc. de Biol., 1903, lv, 656.
22. Abderhalden: Ztschr. f. physiol. Chem., 1913, lxxxvii, 220, 231.

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sites in certain hosts. Examples of this kind might be multiplied, but it is well known that specific foods are essential to the life and growth of both plants and animals. I take it that this means that only certain proteins can be split along the proper lines to serve in metabolism.

The proteolytic ferments of the alimentary canal of man are capable of digesting diversified proteins, but those furnished by the cells of the blood and tissues are more limited in their action. Parenteral proteolytic ferments are of two kinds, non-specific and specific. The former are normally present in the blood and tissue, especially in the former, of all animals. They differ in kind in different species and in amount and efficiency in individuals. Their function is to break up foreign proteins that find their way into the blood and tissues. They are, within limits, general proteolytic ferments as are those of the alimentary canal; though the variety of proteins on which they can act is more limited. They constitute the most important factor in racial and individual immunity. Man is immune to most bacteria, not because they do not elaborate poisons, for every protein molecule contains its poisonous group, but because they are destroyed by the general proteolytic enzymes as soon as they enter the tissue and before they have time to multiply. Such is the leukoprotease of the polymorphonuclear cells.

It has been shown by work in my laboratory that all proteins, bacterial, vegetable and animal, contain a poisonous group. During enteral digestion this group is, partially at least, set free, as is shown by the poisonous effect of peptone when injected intravenously; but this poison is not readily diffusible and the body is protected by the walls of the alimentary canal and a further step in the process of digestion breaks up the poison into harmless split products. When this poison is set free by parenteral digestion there is no such protection offered.

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Jobling and Peterson[23] have shown that blood-serum from which the antitrypsin has been removed or neutralized is poisonous to homologous animals. In this case the blood-serum digests the proteins normal to the animal and the poison thus set free manifests its effects.

Jobling and Bull[24] find that leukoprotease digests typhoid bacilli *in vitro* with the liberation of the poison. They say:

> Freshly washed, unheated typhoid bacilli intravenously injected into dogs cause the development of definite symptoms as early as twenty minutes after the injection. Boiling for ten minutes does not destroy the toxic effects of a freshly washed bacterial emulsion. Complete solution of the bacteria (in dilute alkali) of a fresh emulsion does not prevent the removal of the toxic substance with the coagulable proteins. The action of leukoprotease splits the toxic substance to a noncoagulable state, the digested mixtures being toxic after removing the coagulable portion. The mere presence of the leukocytic ferment is not responsible for the toxicity of the filtrate from the digested mixture, and continued digestion destroys the toxicity of a previous toxic mixture. From these observations it is concluded that the toxic properties of freshly washed typhoid bacteria are not entirely due to toxic bodies that are stored in the bacterial bodies, but that these properties are due largely to products formed by hydration of the bacterial proteins through the agency of ferments present in the circulation of the animal previous to the injection, or which become mobile subsequent to the entrance of the foreign bodies into the blood-stream. Since leukocytic ferments can attack the bacterial proteins *in vitro,* it is possible that the leukocytes are a source of the ferments which are active in experimental and natural cases of intoxication with the whole bacteria.

Thiele and Embledon[25] find protein split products in the blood of animals dying of acute infection and also that the blood-serum of such animals is fatal to healthy ones when injected intravenously. Such observations seem to show quite conclusively that in the infectious diseases the invading organisms are digested, and among the digestive products there is at least one highly active poison.

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23. Jobling and Peterson: Jour. Exper. Med., 1914, xix, 480.
24. Jobling and Bull: Jour. Exper. Med., 1913, xviii, 453.
25. Thiele and Embledon: Ztschr. f. Immunitätsforsch., 1914.

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THE RELATION OF PARENTERAL PROTEIN DIGESTION TO IMMUNITY AND DISEASE

One of the most important facts developed in the study of protein sensitization or so-called anaphylaxis is that the nature and activity of cellular ferments may be modified and specific ferments developed. For instance, work in my laboratory[26] has shown that the blood-serum of a fresh guinea-pig has no digestive action *in vitro* on egg-white, while the serum of an animal sensitized to this protein does digest and lead to the liberation of the protein poison. The same is true of animals sensitized to other proteins, bacterial, vegetable and animal, all of which contain the protein poison. This offers a simple and satisfactory explanation of certain forms of immunity both natural and acquired. A man has yellow fever and recovers and is then immune to this disease. During the attack, the body-cells become sensitized and elaborate a specific ferment which destroys the virus of the disease. When subsequently the man is bitten by an infected mosquito the specific ferment destructive to this virus is promptly active, the virus is destroyed before it can multiply sufficiently to disturb health and the man is immune. The virus of vaccine, an attenuated form of the small-pox virus, is inoculated into the body, sensitizes the cells and subsequently there is for a time at least immunity to this disease. A similar explanation suffices for vaccination for typhoid fever and other diseases for which effective vaccines have been discovered.

The study of protein sensitization has done much to improve our understanding of the phenomena of infection. The pathogenicity of a given virus is determined largely by two conditions. First, the virus must be able by means of its enzymes to feed on the proteins of the animal's body. This condition determines whether or not the virus may be pathogenic to a given

26. Vaughan, Vaughan Jr. and Wright: Ztschr. f. Immunitätsforsch., 1912, ix, 673.

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species. Even close kinship may exist between two species and a given virus be pathogenic to one and harmless to the other. Thus, the ordinary sheep is susceptible to the anthrax bacillus, while the Algerian variety is not. Second, the secretion of the body-cells must not immediately digest and destroy the invading organism. The small-pox virus is pathogenic to the man unprotected by vaccination or by a previous attack of the disease. To the protected man this virus is non-pathogenic because the enzymes supplied by the body-cells destroy it.

When a virus is able to feed on the proteins of the host, and the latter is not protected from the former by efficient destructive agencies, the virus multiplies within its host, converting the proteins of the latter into those of its own structure, and this continues until the body-cells becoming sensitized elaborate ferments which digest and destroy the foreign protein. This process marks the active stage of the disease. When the cleavage action of the enzymes of the body-cells proceeds too rapidly the protein poison is set free in amount sufficient to endanger life. This is a brief and incomplete statement of the relation of cellular digestion to immunity and disease.

We are only on the threshold of these studies. What further investigations may develop no one can predict. Undoubtedly theories will be modified by the further elucidation of observed phenomena and those at present held may be radically changed by the discovery of new facts.

I have recently[27] written fully on the phenomena of infection.

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27. Vaughan, Victor C.: The Phenomena of Infection, The Journal A. M. A., Feb. 21, 1914, p. 583; Protein Split Products in Relation to Immunity and Disease, Philadelphia, Lea & Febiger, 1913.

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LIMITATIONS OF THE DIALYSIS METHOD AS A PRACTICAL TEST FOR PREGNANCY

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When Abderhalden first announced that he had discovered a laboratory method of detecting pregnancy based on the fact that a pregnant woman's serum has the power of digesting human placenta *in vitro,* the announcement was received by the medical profession with remarkably little skepticism. One reason for its ready acceptance in good faith was the recognized high standing of Abderhalden and the vast amount of work he had done on protein metabolism. A second and more important reason was that the medical world had already been made familiar with the basic principles involved in the Abderhalden test through the work of numerous investigators during the preceding ten or fifteen years on parenteral digestion, protein sensitization, and anaphylaxis. Theobald Smith's phenomenon, Pfeiffer's phenomenon, the work of Richet and von Pirquet, and the studies of Vaughan on protein split-products, all really anticipated the discovery of serum diagnosis, just as others before Columbus argued that the earth is round. In a recent controversy over the question of priority, Hamburger claims to have published the basic facts of serodiagnosis in 1903. To this Abderhalden replied that Huppert in 1896 published essentially the same line of argument. That the principle of serodiagnosis of pregnancy is theoretically correct is evidenced by its immediate and universal acceptance as reasonable and probable, and

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the wonder grows that someone had not thought of it sooner; for on *a priori* grounds, what is more logical than the inference that the maternal blood-stream, invaded by solid or soluble placental products, will develop ferments which are choriolytic? That the mother's serum does actually contain such ferments, capable of digesting parts of the cooked human placenta *in vitro,* must now be accepted as a fact proved by thousands of tests. As to the absolute specificity of these ferments for placental proteins, there is still some doubt in the minds of a few competent observers like Pearce and Williams, Kämmerer, Flatow, Fried, Plaut, and others. Inasmuch as the last word has not been said on this phase of the subject, the question as to whether the ferments are absolutely specific for placental proteins, must in my opinion, be left open for the present, notwithstanding the protests of Abderhalden and his co-workers to the contrary.

With the exception of Jellinghaus and Losee in New York, Schwarz of St. Louis, McCord of Detroit, and half a dozen others, the number of experimenters in America who have done extensive work on the serodiagnosis of pregnancy is insignificant, while in Germany and Austria the amount of work done along this line has been prodigious. Unfortunately, some reports of work done on the pregnancy test, especially the earlier ones, must be rejected as valueless, because in some instances the wish has been so obviously the father to the thought that the author has proved whatever he set out to prove.

I have been much interested in trying to discover why so many American workers of known ability have abandoned the test after a brief and unsatisfactory experience with it. Is the test really worthless, or is the technic too difficult for the ordinary trained laboratory worker? A majority of experimenters to whom these and similar questions have been put, honestly believe the test is too unreliable to be of any practical value. It is my opinion that some of the

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workers were influenced to lose their enthusiasm for the test by the reflection that, after all, the making of a positive diagnosis of early pregnancy is very seldom of vital importance to the patient, as it might be, for example, in carcinoma and syphilis. "Time will tell" now, as it did for our grandfathers in medicine, whether or not the woman is pregnant. This would truly be a lame excuse for indifference on our part to a laboratory proof of pregnancy. Until the laboratory test has established a better claim to our confidence, however, American clinicians, it is maintained, can not be expected to display much enthusiasm for it, and they will continue to rely on previously known signs and symptoms, in arriving at an early diagnosis of pregnancy.

It would be manifestly improper here to go into details as to the technic of the dialysis test. Those interested are referred to the numerous original communications of Abderhalden himself, or to the excellent descriptions of the technic of the test by Jellinghaus and Losee, and by Schwarz, which are perhaps the best descriptions of the test published in English. There are, however, a few minor points of technic which in my experience are sufficiently important to justify special mention. They are as follows:

1. If the dialyzing thimbles are threaded with a fine stiff bronze aluminum wire, instead of the silk thread usually recommended, they can be handled with much greater facility.

2. Since the dialyzing tubes are not uniform in thickness, it is well to mark the different tubes with indelible ink or pencil in such a way as to indicate their relative permeability to peptones.

3. Sterile cork stoppers for the containers are superior to cotton plugs, as they prevent the evaporation of the chloroform and toluene at incubator temperature.

4. The principal control should always contain the inactivated serum from the patient's own blood

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rather than that from another patient. This point has been strongly emphasized by Abderhalden himself.

5. Boiling alters the permeability of the dialyzing tubes. I have found that "pasteurizing" them in water at about 180 F. for two or three minutes is satisfactory.

6. Whatever the method used in preparing the placenta, the point of great importance is to see that the placenta preparation contains no dialyzable substance reacting to ninhydrin.

7. Finally, the test and control must be treated exactly alike (except, of course, that the latter contains inactivated serum). It is of less importance to use just so much water, placenta, serum, and reagent, than it is to use exactly the same amounts in both test and control. In making the final tests, I always boil both diffusates simultaneously, over the same flame, at the same rate, and for the same length of time.

The importance of this last precaution will be appreciated when we consider that the final test is a relative one, based in some instances on intensity of color, and, therefore, the test and control must be treated exactly alike if the result is to be at all trustworthy.

Bearing in mind the preceding precautions, and also the other points of technic advocated by the author of the test, I have within the past fifteen months carried out the test on ninety-five subjects, about seventy of whom were women known to be pregnant. Nearly all these tests were made myself in the laboratories of the Marquette University School of Medicine. This is a very small number of tests when compared with the hundreds made at the New York Lying-in Hospital, and some of the continental clinics. My series, however, has at least the advantage of having been begun late enough to enable me to profit by the mistakes of the earlier experimenters, and to avoid all that groping in the dark which made utterly unreliable

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all the early statistics on the dialyzation test. Without giving a complete tabulation, my results may be briefly summarized so as to bring out the two conspicuous features as follows:

First: The pregnant women of the series practically all gave positive reactions. In fact, the last fifty pregnant women of the series all gave positive reactions except one patient who was about two weeks pregnant (as proved by an abortion about two months later).

Secondly: In this series about 12 per cent. of non-pregnant persons gave positive reactions. These included several patients just operated on for acute or chronic appendicitis, pus-tubes, fibroids, and ovarian cysts.

The positive reactions in non-pregnant patients were at first attributed to errors of technic. Possibly that is the true explanation. But with the most painstaking care I have not been able materially to reduce the percentage of non-pregnant patients giving positive reactions. According to the results obtained in this series, it would appear that the test is of value in a neagtive sense only. That is to say, if a woman does not give a positive reaction to the test, it is safe to conclude that she is not pregnant. On the other hand if she gives a positive reaction, we can only say that she is probably pregnant. This limitation largely neutralizes the value of the test as it is now carried out.

Some of the contributory causes which in my opinion have limited the popularity and usefulness of the serum test for pregnancy are the following:

1. Although the test appears on paper to be comparatively simple, its proper execution is exceedingly difficult and time-consuming.

2. The test is not well suited for the general clinical laboratory, but should be made only by one who is doing the test every day, and always has the materials fresh and in perfect condition.

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3. The presence in the blood of filterable substances reacting to ninhydrin has been noted by many observers and writers as occurring during the height of digestion, or in certain condition of altered metabolism. These substances have been referred to as sources of possible error in the dialysis test for pregnancy. This fallacy can largely be eliminated by using for the control the inactivated serum from the patient who is being tested.

4. The presence in normal blood of non-specific proteolytic enzymes possessing feeble powers of digesting proteids parenterally is, I believe, generally conceded. It seems reasonable that these normally present enzymes might, under certain conditions other than pregnancy, cause sufficient digestion of placental albumin to vitiate the test, or at least make the result doubtful.

5. To procure for the test a sufficient quantity of serum entirely devoid of any trace of hemolysis is sometimes a matter of extreme difficulty, especially in those cases in which the blood must be transported for any considerable distance to the laboratory.

6. The use of parchment tubes or thimbles as filters, constitutes one of the intrinsically weak spots in the dialysis test. No matter how well they are made or how positively the manufacturer may guarantee them, we know that the parchment tubes are an unstable and inconstant quantity, and may be a source of well-nigh unavoidable error. Even after the tubes have been thoroughly tested and the imperfect ones thrown out, we have found that their permeability to peptones and amino-acids becomes altered by boiling, which is the only method of sterilizing them perfectly. We hold that no method of carrying out a biologic test can be an ideal one so long as it depends for its results on the integrity and constancy of a frequently boiled parchment membrane. Errors from this source need not be numerous, but the fact that they will occur

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now and then stands as an indictment against the absolute accuracy of the dialysis method.

A year ago I made the prediction before this section that in the course of time the Abderhalden test for pregnancy would take its place with the Widal and Wassermann tests in point of reliability. Further personal experience with the test and an observation of the experiences of other workers have convinced me that that time has not yet arrived.

SUMMARY

The dialysis test for pregnancy in its present stage of development is of value chiefly in a negative sense only; that is, if a woman fails to give a positive reaction, she is not pregnant. If, on the other hand, she gives a positive reaction, we can only say she is probably pregnant; for with our present technic, from 10 to 15 per cent. of non-pregnant persons will give positive reactions.

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VALUE OF ABDERHALDEN'S BIOLOGIC REACTIONS TO THE OBSTETRICIAN AND GYNECOLOGIST

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At the present state of the investiagtion it is possible with the aid of Abderhalden's dialyzation reaction and with his optic method to demonstrate the strict specificity of the so-called protective ferments, demonstrating thereby at the same time that the protoplasms of the various organs and tissues of the animal organism differ sufficiently from each other in chemical structure to be recognizable with the aid of these ferments, even after they have been boiled or after they have been reduced to the peptone stage. It is possible to prepare placental albumin and placental peptone which will be readily cleft by pregnancy serum, but which will not be cleft by other serums and certainly not by serum from a cancer patient; on the other hand, it is also possible to prepare coagulated cancer tissue which will be cleft by cancer serum and which will not react with the serum of pregnancy.

Thus, by making a number of cross-examinations, examining the same cancer serum and the same pregnancy serum against the same placental albumin and the same cancer alubumin in the same experiment, and finding under these condtions that cancer serum reacts with cancer tissue only, and that pregnancy serum reacts with placental tissue only, one proves the specificity of the protective ferments and the specific structure of the various protoplasms. This demonstration is constantly made in Abderhalden's institution; it has been made in a few other laboratories;

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it has been made time and again in the laboratory of the Department of Obstetrics of Washington University; my associates and I have taken great pleasure in making these demonstrations before the numerous visitors, including experienced laboratory men, who have come to St. Louis for the express purpose of studying certain points of technic.

Obstetricians and gynecologists have a certain advantage in this investigation, which should mean that they also have a certain obligation to assist in demonstarting the truth of this new doctrine. In the women's clinic pregnancy cases and cancer cases are always to be found; the material for preparing albumin or peptone can be obtained fresh from the delivery room or from the operating pavilion; the special precautions in obtaining and handling serum can be readily observed; the women's clinic, therefore, must become the training camp for those laboratory men who wish to engage in research work in this new field.

It is a waste of energy and material to attempt investigation of other conditions and to work with other organs and tissues before the investigator has learned the fundamentals of this work and has tested the efficiency of his technic on the touchstone of the cross-examination of pregnancy and cancer.

Be it remembered that in these two examinations the material for the preparation of albumin or peptone can be obtained in perfectly fresh condition; while, when working with other organs and tissues, we must obtain the material from the necropsy room or else we must substitute animal organs and tissues, which substitution is not as yet justified on the basis of convincing experiments. The fact that we can prove that the protective enzymes found in pregnancy and in cancer are specific, does not mean that we are willing to use these biologic reactions for diagnostic purposes without the greatest reserve.

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The serodiagnosis of pregnancy, even in expert hands, is subject to error on account of unreliable dialyzing thimbles; danger from this source is reduced, but not eliminated, by multiplying tests and controls. The optic method eliminates this source of error. We find, however, that peptone obtainable in the market is not always reliable and have therefore started to prepare our own peptone. It is true that the manufacturing chemist can do this work better and cheaper than we can, but we can start with a blood-free placental preparation, which is so essential a point that we are unwilling to entrust this work to the manufacturer; we also need other peptones which are not offered on the market.

Besides this source of error contained in the dialyzation method, it must be remembered that, even when the test works out correctly, a positive reaction simply means that the case in question harbors placental elements; usually it means that the patient is pregnant. Placental elements, however, are often harbored in the uterus or even in other parts of the body for long periods after delivery; this is especially true in cases of hydatidiform mole, in which the retention of syncytial cells is so apt to lead to the development of chorio-epithelioma.

These tumors, being of placental origin, naturally give a positive reaction with pregnancy serum. We have a case under observation, at present, of a woman who passed a hydatidiform mole over three months ago, who menstruates regularly, who is not pregnant and whose serum continues to react positively with placental preparations. It is for these reasons that we are unwilling to consider a positive reaction of serum with placental preparations a convincing proof of pregnancy, except in connection with the clinical history of the case and in connection with a thorough physical examination. Even with these limitations the serodiagnosis of pregnancy has been of real service to us in arriving at a correct diagnosis in a number of perplexing cases.

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In the serodiagnosis of cancer we have likewise found various difficulties. The serum in a suspected case may give a negative reaction although the case is one of cancer; this seems to happen when the cancer tissue used in the test is of different histologic structure from the cancer on which the test is made. We also find difficulty in securing cancer material which can be rendered blood-free and which is free from infection; at other times, it is not possible to isolate the cancer tissue from the tissue of the affected organ. So far it has not been possible to prepare the desirable quantity and varieties of malignant albumins; as regards the preparation of cancer-peptones, this has been simply out of the question.

In the course of time we hope to have on hand coagulated preparations made of the various forms of malignant tumors, but until we are thus provided, we must expect to get negative reactions in cases which differ in histologic structure from the cancer material used in the test.

Eventually, it will be possible to test the serum of suspected cases against mixtures of cancer albumin and cancer peptones and, in case of positive reaction, to ascertain the histologic structure of the tumor under investigation by a second series of tests against the component albumins or peptones of these polyvalent mixtures. The serodiagnosis of cancer will be of considerable value in those cases in which it is not possible to obtain material for microscopic examination before a serious operation is undertaken. The following is a case in point:

A woman, aged 68, suffered with pain and swelling in the region of the gall-bladder, and with jaundice; the diagnosis pointed to malignant disease, but a stone in the common duct could not be entirely excluded and an exploratory operation seemed indicated. The serodiagnosis made the diagnosis of malignancy positive, for the patient's serum reacted positively with cancer albumin and negatively with placental albumin;

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there was no need for removing the patient from a comfortable home and from her family; she died several months later of cancer of the liver.

Serodiagnosis of cancer is of still greater value for controlling cases after radical operations and for watching for a return of disease; also for observing the effect of Roentgen-ray treatment and other therapeutic measures on cancer cells.

The difficulty of securing reliable cancer preparations for this control work has induced us to use the material obtained at operations, regardless of its fitness for general investigations. The serum of the patient is obtained on the morning of the operation; the material obtained at the operation is prepared as well as conditions permit; a small portion of it is used immediately against the patient's serum and gives, as a matter of course, a positive reaction; the rest of this preparation is preserved for the patient's individual benefit and will answer for the control of her own case, although usually unfit to be used in tests on other patients.

This work was started only a few months ago; it is too early to estimate its actual value. This much is evident; the serodiagnosis of pregnancy and cancer by these biologic reactions is already of considerable practical value, but, at present, these methods are not suitable for the routine work of commercial laboratories; the attempt to employ them outside of research laboratories, closely connected with a hospital service, must lead to disappointment and tends to retard real progress in this field.

The demand for easier and quicker methods of serodiagnosis has induced Abderhalden to make the attempt to demonstrate the action of proteolytic ferments by the method used by Gruetzner in demonstrating the action of pepsin. Gruetzner uses fibrin stained with carmin; when some of the fibrin is digested, the carmin is set free, the solution of pepsin is thereby stained pink. My associate, Dr.

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Newell, has used carmin-stained placenta according to Abderhalden's directions, placing about 0.25 gm. of this tissue and 2 c.c. of serum in small test-tubes; after approximately eight hours, the tests containing pregnancy serum turned pink, while the control serum was still yellow, later, as all serums turned more or less pink, it seems worth while to continue these attempts. Abderhalden is also experimenting with other stains, and he advises against the use of this method at present, except in the way of experiment.

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ABSTRACT OF DISCUSSION

ON PAPERS OF DRS. VAUGHAN, ECHOLS AND SCHWARZ

Dr. Herman J. Boldt, New York: I am in accord with what Dr. Echols has said concerning his clinical experience and the conclusions at which he has arrived from his studies. I am not in accord, however, with those who wish to entirely obliterate serodiagnosis, the test of Abderhalden for the purpose of making a diagnosis of pregnancy. That is an unjustifiable position to take, both scientifically and with regard to the work done by those who have paid attention to it. Would any one dare to make a diagnosis of any illness by a single symptom, be that objective or subjective? I believe not. I believe that we should therefore class the dialysis method as only an objective symptom in the chain of symptoms for the purpose of establishing a probable diagnosis, and as such I regard it. Three or four months ago I saw in consultation a woman who was supposed to have an intra-uterine pregnancy. When questioned she gave subjectively a typical history of an ectopic gestation, and objectively this was confirmed. We must remember that between uterine abortion and an ectopic gestation or tubal abortion, it is at times difficult to draw a dividing-line in diagnosis. Subjectively the patient showed the condition of an ectopic gestation, I thought there was a hematocele posterior to the uterus. Since she had no symptoms leading me to believe that she must be immediately operated on I thought it best to keep her under observation. Her condition did not change. For two weeks she was kept at the hospital, when I suggested operating. We found a soft incarcerated subperitoneal myoma, that she had been pregnant and had aborted. The Abderhalden reaction in that instance proved negative. I thought that here was an instance in which we could make the diagnosis by the dialysis method and that it had failed. It did not fail, because we did have

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a negative reaction. We know that the Abderhalden test is always negative a few weeks after expulsion of the fetus. Another case was that of a woman brought by a physician to have her uterus removed. The examination showed the presence of a myoma. She had been seen by a number of colleagues. All advised operation without further delay. The menstrual period had been last seen six weeks previously and she had some gastric disturbance; there was a slight lividity about the mucosa of the vagina. I concluded that there was probably a myoma present in connection with early pregnancy. The Abderhalden test gave a positive reaction. The woman was extremely anxious to have offspring. They had been married fifteen years. In connection with the subjective symptoms I believe I had a right to advise her to await conditions. Under these circumstances we have no right to discredit such a scientific sign of diagnosis if we regard it as only one of the conditions in connection with the case.

Dr. Charles S. Bacon, Chicago: The interest in this subject is not only that due to the question of diagnosis, but to the broader question of ferment digestion. Experiments in the University of Illinois comprise work done by the research fellow in this department. Our results conform almost exactly to those obtained by Dr. Echols, and we cannot agree with Dr. Schwarz in finding a specific ferment in these cases. In the number of observations made care is taken to follow Abderhalden's method exactly. The care that we have taken in the control and the amount of material we have used in making observations on other classes of cases make our conclusion worthy of consideration.

Dr. Philip F. Williams, Philadelphia: We have been working with the Abderhalden test for pregnancy at the University of Pennsylvania for the past two years. In our earlier work we confined our efforts to the dialysis method in pregnancy. Every pregnancy serum examined gave a positive reaction, but a number of serums from non-pregnant patients also responded positively. When we used uterine carcinoma tissue as substrate we found a digestion of this tissue as well as of placenta by the pregnancy serums. As the dialysis shells were found to be unfit for use after the preliminary tests in more than 50 per cent. we devised a method of coagulation and filtration. By this we also had an occasional positive reaction in a non-pregnant serum. Our animal experiments consisted in the injection of kidney tissue into dogs and rabbits. After a single injection, ferments were developed which digested liver tissue as well as kidney tissue; after multiple injections there seemed to be a tendency for a more definite digestion of kidney tissue. The commercial placental peptone was used with pregnancy

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serums in the polariscopic method. Our poor results may have been due to the peptone, for in but few cases did we obtain a change of more than 0.04 degree. We have tried to measure the amount of amino-acid in a mixture of pregnancy serums and various placental preparations, before and after incubation, using the Van Slyke deaminization apparatus, but have not obtained any definite data at present. In reviewing our work of the past two years we cannot feel that the pregnancy ferments are of a specific nature.

Dr. F. H. Falls, Chicago: In our work with the Abderhalden test in the University of Illinois we followed the technic absolutely as given by him. We found that fully 12 per cent. of normal persons gave a positive reaction, following exactly the same technic used in examining the pregnant patients. That led us to examine the serum of various pathologic conditions and in these also we found many positive reactions. It seemed important to find what pathologic condition would give positive Abderhalden tests. We investigated cases of pneumonia, nephritis, splenomyelogenous leukemia, acute articular rheumatism and other conditions. Many gave positive Abderhalden reactions when exactly the same technic was used as in the pregnancy test with the placenta as a substrate. This will to a certain extent vitiate the reliability of the Abderhalden test. I think the technic is not too difficult for a person to develop and use who has had experience in the serologic work. I might say that, so far as the cancer problem is concerned, it has been my experience that serum from cancer patients will digest placenta as a substrate. This is not true in every case, however, and there seems to be no definite relation between the extent of the cancer and the digestion that the serum will give. Concerning the boiling of the dialysis thimbles as given by Dr. Echols, we found that boiling No. 579 Schlicter thimbles for two minutes did not cause permeability of the thimbles within a reasonable length of time. I used the thimbles for a month or so and tested them repeatedly, and in no case was there the slightest trace of color. After using a set of thimbles for three months they were discarded, not because they let through albumin, but because their equivalent permeability to peptone had been altered. I think it important to run the inactivated serum control. Only so can we judge the reaction. If, as Dr. Echols said, the inactivated serum-control was negative in cases taken at the height of digestion, then it cannot be the amino-acids present in the blood, but ferment activity in the active serum that accounts for positive reactions under these conditions.

Dr. Charles Goodman, New York: During the latter part of last year it was my privilege to learn in Abderhalden's laboratory the technic of the dialysis test described by him. It was my intention to employ the test in seeking a method

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of overcoming autolysis in homotransplantation of organs. In a series of transplantations in fourteen dogs we were able to demonstrate the presence of a ferment capable of digesting suprarenal tissue in the presence of a transplant of the thyroid gland. In a series of cases of pregnancy, my assistant, Dr. Berkowitz, and I were able to demonstrate the presence of a specific ferment with a uniform degree of success by the Abderhalden dialysis test. In a series of cases of malignancy we have been able to demonstrate the presence of a specific ferment capable of digesting carcinoma or sarcoma in thirty-one out of thirty-two instances. We always used serum thimbles as a control. A negative result is of more value than a positive reaction. The test is exceedingly delicate, and false and divergent results may be obtained owing to slight errors in technic. The technic is complicated and difficult but not beyond the realm of possibility of being mastered by serious and diligent application. The fundamental rules of the test must be obeyed. We must not condemn the method because it requires careful working. Professor Abderhalden advised me and the other workers, after having made use of the thimble, to cleanse it in running water for thirty minutes and then drop it into boiling water for fifteen seconds and no longer, because if it boils it becomes thick. After the thimbles have been in boiling water for fifteen seconds they are put into a container of sterilized distilled water and toluene and kept for use.

Dr. Michael G. Wohl, Philadelphia: We forget that Abderhalden's reaction has its restrictions and we demand too much from it. The apparent simplicity of the test is responsible for the great inaccuracy of the results obtained through not paying strict enough attention to its technic. Among the errors pointed out I wish to emphasize the importance of boiling the dialysate. The reaction is more quantitative than qualitative. A definite amount of protein cleavage products present in the diffusate combine with a definite amount of ninhydrin giving the violet color. Now, if there is less of the protein cleavage products or less ninhydrin, you will either get a faint reaction or none. The unequal boiling, therefore, whereby you obtain in one instance a greater concentration of the diffusate, and in another a lesser, will be responsible for the divergent result. We count one minute from the time bubbles appear in the test-tube. The amount of water added to the beaker outside of the thimbles must always be uniform (20 c.c.). As Dr. Echols said, it is not necessary to pay much attention to the quantity of water added to the outside of the thimbles. I believe that this may account for the discouraging results. I hope that soon we may have a uniform color-scale by which each worker may determine the end-results of the reaction. On the specificity of the ferments the future of the reaction

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depends. From our work with this reaction in nervous diseases in the neuropathologic laboratory of Dr. Weisenburg and in the Samaritan Hospital, I believe that the reaction is organ specific.

In our cases we find that serums of male patients with dementia praecox digest testicular tissue, and serums of female digest ovarian tissue. Is this not sufficient evidence of the specificity of the ferments? True, we may, under certain conditions, get cleavage of placental tissue or of any tissue with serums of non-pregnant patients, when there is a leukocytosis. As is well known, leukocytes contain non-specific proteolytic ferments and, consequently, serums from patients with an inflammatory condition might give a positive reaction when a negative reading was expected. We make a differential blood-count in all such cases. Another possibility of error lies in some hemorrhage, bruise or hematoma whereby through the hemolysis of the red cells a ferment is liberated which might possibly cause cleavage of protein substances. Do not condemn the reaction in an offhand manner if it has not met your expectations. Realize the qualitative nature of the test. Search for the cause of your wrong reading. Because you get the positive Wassermann in yaws or psoriasis you do not necessarily discard the reaction but govern yourself in accordance with the clinical picture. Why not apply the same rule to the Abderhalden?

Dr. Clarence F. Ball, Rutland, Vt.: I contradict the statement that cancerous and pregnant conditions will interact generally. I have a series of nearly 100 cases in only a very few of which has pregnancy given a cancer reaction or cancer a pregnancy reaction. Some of the cases have come to operation and I have not been able to demonstrate third-degree proliferation of the epithelium; there was a second-degree proliferation, however. My work in all of these cases has been done running four thimbles: one with activated serum, one with the serum and cancer tissue, another with either serum alone or the tissue—whichever I suspected, and the fourth with placental tissue. I use the cancer tissue for the primary test; placental for the secondary fundamental. The question of the validity depends on more than 90 or 900 cases: it depends on work which must be as extensive as that on the Wassermann has been. It may share the same fate as the Wassermann, or even worse. I believe, however, that there is a better future for the test. I have followed Dr. Schwarz' modification of the Abderhalden test, although I have not been to Dr. Schwarz' clinic. As to the question of cancer fundaments, obtain all your cancer tissue with as many cells as you can obtain free from the tissue invaded. The tissue that I have had the best results with has been that from a metastasis in which practically all of the tissue was cancer cells.

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Dr. O. Lowy, Newark, N. J.: I have done considerable work in dialysis and have found that, as Dr. Wohl has brought out, the question of boiling is very important. To do away with some of the uncertainty I boil my solutions in a water-bath and instead of boiling for one minute I boil them for ten, using the four-thimble method and the activated serum. At the end of ten minutes I read the result. In using the flame a different heat is obtained according to the position in which the test-tube containing the serum is held, whether toward the center or end of flame. Another method to which I resorted to overcome error is: in a positive result I retest that same thimble at once against albumin. If the albumin gives a positive result my test is of no value. If it is negative I test against peptone. If that is positive I know that my test is right. As far as the value of the test is concerned, only the cumulative evidence of thousands of cases will do that. Up to the present we have tested 150 to 180 cases of cancer. In a number of cases of diabetes I have obtained a positive result; why I do not know.

Dr. A. J. Carlson, Chicago: There is a fundamental discrepancy between one of Professor Abderhalden's statements and one of the main points in Dr. Vaughan's paper. Abderhalden takes the position that normal blood contains no polyvalent proteolytic enzymes, while Dr. Vaughan maintains that "the blood is a digestive fluid." Just there lies the main source of error in the practical utility of the Abderhalden test. I have had no personal experience with this test, but I have done some work on the ferments of the blood. I cannot accept Abderhalden's dictum as to the absence of polyvalent ferments in normal blood. I predict that normal blood will be found to contain polyvalent ferment or ferments, that when specific proteins are liberated into the blood, specific ferments are produced. That means that the Abderhalden test must be, not a qualitative, but a quantitative one. Of course I admit that the question whether or not normal blood-serum contains polyvalent ferments is a difficult one to decide. We have no means of obtaining serum identical with the plasma of the circulating blood. Dr. Vaughan emphasizes particularly that the leukocytes and the blood platelets are the source of some of these ferments. That may be partly true, but I wonder whether he is not unduly influenced by the distinguished gentleman across the water — Metchnikoff. In regard to many of the immune bodies I am confident on the basis of work by Dr. Hektoen and myself that tissues other than the leukocytes are the most important.

Dr. Henry Schwarz, St. Louis: I wish to say, since a great many men are influenced by the technic which I had given out, that there is one point about boiling the thimbles which was incorrectly stated in my paper read at Providence, R. I. I there stated that I boiled the thimbles five or six

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minutes. I do not. Such boiling spoils the thimbles, rendering them impermeable. We simply dip them in boiling water for not more than fifteen seconds, as Abderhalden suggests. A great many men with just as much training as Dr. Falls undoubtedly has, and with similar experience, have found when they published their results and their methods, that some little point of technic was wrong, which upset the whole matter. We are all willing to admit that the thing is not quite ready for everybody to work with; we are not quite ready to advise serodiagnosis of pregnancy or cancer, on a commercial basis at any price. We would not take any money for it, but we are all working in the same field and we are trying to make good. There are others who are not obtaining convincing results. Come to St. Louis, we shall be glad to show you in our laboratories the work that we are doing.

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NEWER POINTS OF VIEW REGARDING THE PART PLAYED BY DIFFERENT FOOD SUBSTANCES IN NUTRITION

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For the continued performance of their physiologic functions and for the maintenance of their material integrity, living organisms are dependent on an adequate supply of suitable nutrients. Their needs in this respect are evidently not uniform at all times. Periods of growth and development, of convalescence and repair, determine special requirements that are not encountered at other epochs in the life-cycle, just as rest and activity alternate in making unlike demands on the organism during the routine of each day. It was realized, long ago, that these varied manifestations of the bodily mechanism may create specific demands in respect to the food substances; but it was a forlorn hope to expect to penetrate far into the mysteries of nutrition until some definite and fairly comprehensive knowledge regarding the chemical make-up of the products taken into the body as food had been unveiled. An elementary understanding of the fate of the fats and carbohydrates came earliest, because these foodstuffs were the first to have their chemical constitution unraveled. The story of the metabolism of the protein substances is only now beginning to keep pace with the newer knowledge of the structure of these highly complex nitrogenous compounds, and there are chapters bearing on numerous other factors in the food intake — on the place of the inorganic

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salts, the lipoids and diverse accessory features in the dietary — that still remain to be written even in their broadest outlines.

To speak of "newer" points of view regarding the rôle of the different food substances in nutrition, as I am expected to do, implies a contrast of to-day with yesterday in this progressive domain of physiology. One need not turn back far to reach the days of Liebig, to whom, above all others, are due the beginnings of present-day knowledge of the constitution of foods, tissues and excreta. In Moleschott's popular "Physiologie der Nahrungsmittel," published in 1859, the chief concern of nutrition was portrayed as the production of good blood. He wrote:

> One of the foremost questions which humanity will always address to the physician, is how good, healthy blood, capable of development, can be attained. And, modify the inquiry as they will, all those who are concerned therewith are forced by experience to admit, whether it be frankly or with reserve, that our thoughts and our loves, our children and our powers depend on our blood—and our blood on food.

Accordingly, Moleschott further maintained that vigorous nutrition is the consequence of efficient formation of blood; so far as the latter process means an enrichment of the circulating fluid in protein substances, the vigorous nutrition would express itself, above all, in the muscular tissues. "The more meats, legumes and cereals are consumed and digested," says Moleschott, "the plumper are the muscles, the firmer their fibers, the more powerful their action." Other diets, of rice, or vegetables, or fish, were pictured as leading to an enfeebled musculature. For the fats and carbohydrates there were known depots other than the muscles. Not until the work of Carl Voit was the principle of nitrogenous equilibrium definitely established for the animal body.

The vague and indefinite generalizations of a generation ago in respect to the functions of the foodstuffs have slowly been transformed into the more

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tangible theories and suggestive hypotheses of the passing decade. To give you some more concrete ideas of what these changes have involved I may refer to the teaching current in my own student days, more particularly as it is related to nitrogenous metabolism. Sir Michael Foster wrote in 1891: "At all events the greater part of the proteid material of the food enters the blood as proteid material, either as peptone or in some other form, and is carried as proteid material to the tissues." The latest dictum respecting this phase of absorption presents an entirely different point of view. Proteins are now understood to be disintegrated in the digestive tract into relatively simple nitrogenous fragments, the amino-acids, not exceeding twenty in number. It is these digestion products, absorbed into the blood-stream and now demonstrated beyond peradventure to be present there, that represent the real form in which the protein intake of the body is transported throughout the organism. Protein metabolism has at length become essentially a question of the behavior of amino-acids in the body — their uses and fate.

So far as the nitrogenous transformations are concerned, the amino-acids are the center of interest in nutrition, the pivot about which the modern conceptions turn. For him who would understand the real significance of the proteins in the functions of the organism there is no escape from the necessity of appreciating clearly the nature of the amino-acids — those derivatives which arise when, either in the laboratory of the chemist or in the alimentary processes of digestion, albuminous substances are disintegrated. A generation ago it was the superficial distinctions between animal and vegetable proteins, between albumins and globulins and albuminoids that formed the basis for discussions of relative nutritive merit. To-day we are more concerned with the question whether this or that protein, whatever its biologic origin, will yield the characteristic, desired amino-

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acids, such as tyrosin and tryptophan, leucin and lysin, glycocoll and cystin, histidin and arginin. Our attention is fixed on the building-stones or units out of which the great protein structures are put together.

Instead of referring to the proteins in terms of their physical properties or empirical composition — their content of carbon, hydrogen, oxygen, nitrogen or sulphur — at least so far as the problems of nutrition are involved, the time has arrived for estimating their behavior in the organism on the basis of the quota of each of about eighteen well-defined amino-acids which the individual representatives of this group of foodstuffs can yield. Most, if not all, of these amino-acids are essential for the construction of tissue and the regeneration of cellular losses. In proportion as any specific protein can furnish these constructive units it may satisfy the nutritive needs of the body. The efficiency of the individual protein in this respect must depend on the minimum of any indispensable amino-acid that it will yield; for it is now known that some of them cannot be synthesized anew by the animal organism. If, for example, a protein or mixture of proteins comparatively deficient in their yield of the sulphur-containing amino-acid cystin be furnished alone to supply the body's nitrogenous requirement, the production of new, cystin-yielding molecules of protein will be limited by the amount which is available in the diet. An excess need not be wasted, for it can be burned up like sugar or fat to provide energy; but new construction or growth is limited by the minimum of the essential unit.

Although a supply of all or most of the characteristic amino-acids derived from typical proteins is essential for the construction of new tissue or the repair of the old, it by no means follows that all of them are inevitably needed in the ordinary function of maintenance, that aspect of nutrition characterized by the preservation of unchanged body-weight in the adult from day to day. Nevertheless, certain amino-

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acids are absolutely indispensable even in maintenance. This is well shown in recent observations conducted by Osborne and myself under the auspices of the Carnegie Institution of Washington. We have succeeded in keeping small animals (albino rats) during long periods on mixtures of isolated protein, fats, carbohydrates and inorganic salts as they are present in the protein-free fractions of milk. The advantage of this experimental procedure lies in the fact that it is possible to test, as we have done, the nutrient efficacy of individual proteins. The upshot of our now extensive experience has been to demonstrate pronounced differences in proteins from different sources. Some, like the proteins of milk, the edestin of hemp-seed, the albumin of egg-white, are individually capable of satisfying the nitrogenous needs of the body at all stages of its development. Chemical analysis has disclosed the potential existence of most of the familiar amino-acid groups in each of these protein complexes. When the gliadin of wheat, a prominent protein of this seed, is fed as the sole protein, adult animals are suitably maintained; but growing animals cease to increase in body-weight, remaining in nutritive equilibrium without growth, unless the amino-acid lysin $\begin{array}{l}CH_2.CH_2.CH_2.CH_2.CH.COOH\\ \quad\;\;|\qquad\qquad\qquad\qquad\;\;|\\ \quad NH_2\qquad\qquad\qquad\quad NH_2\end{array}$ is added to the gliadin-food. Thereupon growth is promptly resumed. The explanation becomes apparent in the fact that gliadin is almost entirely devoid of the lysin radical; and inasmuch as this is obviously needed for new protein construction, growth cannot proceed until the missing unit is supplied. Again, zein, the most conspicuous protein of the maize kernel, fails to yield either lysin or tryptophan $\begin{array}{l}\quad\;\;C{-}CH_2.CH.COOH\\ \quad\;\;/\;\;\|\qquad\;\;|\\ C_6H_4\;\;\;\;CH\;\;\;NH_2\\ \quad\;\;\backslash\;\;/\\ \qquad\;\;NH\end{array}$ or glycocoll $\begin{array}{l}CH_2.COOH\\ \quad\;\;|\\ \quad NH_2\end{array}$ and accordingly is entirely inadequate to meet the nitrogenous needs of animals in respect to either maintenance or growth. It may be fed in the greatest

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abundance; yet the animals decline in health unless the zein is supplemented by some more perfect protein. If the amino-acid tryptophan is added to the imperfect maize protein, maintenance of body-weight, without growth, is promptly established. Evidently tryptophan is indispensable for some physiologic function quite apart from increase in tissue substance. If both tryptophan and lysin are added to the zein, the diet thereupon becomes suitable for growth. In glycocoll, likewise missing in zein as in several other proteins, we have an instance of an amino-acid which can undoubtedly be manufactured when it is needed. From these facts it is apparent how essential certain amino-acids are in the nutritive processes and how unable the body is to synthesize some of these anew.

I trust that this hasty review has given emphasis to the contention that the problem of protein metabolism has largely become one of amino-acid supply. Provided only that they are rendered free and available by the digestive processes, the body cares little whence its amino-acids are obtained. Meat proteins, egg proteins, cereal proteins—all serve in the same sense. Indeed, the amino-acids themselves, isolated in the chemical laboratory, appear to be equally available. We begin to understand why a diet largely made up of corn with its abundance of the imperfect zein and its relative poverty in more complete proteins may be poorly suited for growing individuals; why corn is so advantageously supplemented by a little skimmed milk with its casein and lactalbumin; why gelatin, which does not yield tyrosin, tryptophan or cystin, fails by itself to meet the demands of the body for nitrogenous pabulum; why a low intake of a suitable protein may be infinitely more advantageous to nutrition than a surfeit of an "incomplete" protein.

Nor is the story of the rise of the physiology of the amino-acids ended here. These compounds furnish a superior medium for the growth of bacteria, not infrequently yielding products of pronounced toxicity or

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pharmacologic potency. A series of amins ranging from simple forms to complex types owe their origin to the action of micro-organisms on proteins, both outside of the body and within the alimentary tract. Putrescin, cadaverin, tyramin, histamin are derived from the amino-acids arginin, lysin, tyrosin and histidin, respectively. Likewise the widely vaunted alimentary offenders, indol and skatol, are chargeable to the putrefaction of the tryptophan group in the protein molecule; phenol and cresol are similarly derived from tyrosin. Thus many of that vague and heterogeneous collection of compounds once convincingly included in the indefinite expression "ptomains" are doubtless nothing else than the fragments of amino-acids that have fallen sacrifice to bacteria before they could be absorbed intact into the blood-stream. Now that the production of these more or less toxic amins and alcohols is clearly referable to the action of known types of bacteria on individual amino-acids, the way to avert the malaise or more serious consequences with which the putrefaction products are so often charged may be a step nearer. Gelatin, for example, furnishes no tryptophan, tyrosin or cystin; the intestinal bacteria are therefore unable to liberate indol, phenol, hydrogen sulphid or mercaptans from it.

To still another domain the discovery of the amino-acid structure of the proteins has admitted fresh rays of light. The phenomena of immunity, creating almost an entire new science of immunology; the precipitin reactions; anaphylaxis and its varied manifestations—all of these point to the participation of proteins; and, what is more significant, owing to the highly specific character of the responses, they postulate the existence of individual proteins in numbers far exceeding the possibilities suggested by the ordinary criteria of individuality. How are we to conceive of a hundred or a thousand specific albumins from as many tissues

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and individuals? We need merely grasp the convenient hypothesis furnished by our knowledge of the amino-acids which they yield. Assuming that ten different amino-acid derivatives are united even without duplication to form a single protein, mathematical calculation indicates the possibility of over three million different combinations; for eighteen amino-acids the possible combinations have been estimated to exceed millions of millions. In the face of such figures one realizes what specificity may really mean when thousands of structurally isomeric compounds can theoretically, at least, yield precisely the same proportions of the same amino-acids and yet remain distinct in respect to the relative arrangement of their constituent units. The development of an almost innumerable number of special enzymes, as suggested by Abderhalden's recent studies now widely applied in serodiagnosis, likewise finds the possibility of an explanation in what has just been noted. A thousand structures may require a thousand different keys to unlock them.

It might be illuminating to dwell further on other consequences entailed by the development of the biochemistry of the amino-acids; but we must return again to the physiology of two decades ago for a reminder of other changes that have been wrought by the hands of progress. Urea was, of course, recognized as the predominant excretory end-product of nitrogenous metabolism. Its history was difficult to follow, and almost entirely obscured. Foster voiced a then current belief in his widely studied text-book in suggesting that a large portion of the urea leaving the body by the urine is formed from the creatin of the muscle, though the objections to such a theory were already obvious to him. The normal presence of creatinin in the urine offered a difficulty which was not entirely solved by charging its origin to creatin ingested as meat. Nor was only creatinin left as a

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source of confusion. Uric acid and other excreted products remained to be accounted for, as they occurred in varying amounts in the urine.

Foster wrote:

> Urea is the chief end-product of proteid metabolism, but that end is probably reached in several ways; so that probably a very large number of nitrogenous chemical substances make a momentary appearance in the body. Some of these fail to become urea, and either without or after further change make their appearance in the urine. But we do not know whether their appearance is accidental, the result of imperfect chemical machinery; or whether they, though small in quantity, serve some special ends in the economy. Perhaps sometimes, or with some of them it is the one case, at other times or with others it is the other case. . . . The evidence as far as it goes tends to show that the metabolism of proteid is very complex and varied, that a large number of nitrogen-holding substances make a momentary appearance in the body, taking origin at this or that step in the downward stairs of catabolic metabolism and changing into something else at the next step, and that the presence in various parts of the body and even in the urine, in small quantities, of so many varied nitrogenous crystalline substances, forming a large part of what are known as extractives, has to do with this varied metabolism. Possibly the transformations by which nitrogen thus passes downward take place to a certain extent in such organs as the liver and the spleen, which are remarkably rich in these extractives. But the whole story of proteid metabolism consists at present mostly of guesses and of gaps.

In this veil of mystery that surrounds the chemical transformations of the food-substances in the body there are today many rifts through which new truths have been brought to light. Uric acid is no longer associated with the metabolism of the simple proteins, but is recognized as the excretory end-product of the purins, a group of compounds distributed widely in nature, either free or combined as they are in the nucleoproteins of cellular tissues. By the exclusion of glandular material, like liver and sweetbreads, and of meat with its free hypoxanthin, the dietitian can reduce the production and output of uric acid in man at will. Here is one of the striking instances of where, through the newer knowledge of foods and metabo-

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lism, the physician has become a master of the chemical performances of the organism. The creatinin of the urine, provided that it is not reinforced by ingested precursors in meat, represents a story of the inner workings of the organism quite independent of the protein in the diet. The ammonia output is in a measure an index of the overproduction of acids in the body. Although by no means the only regulatory device whereby the neutrality of the blood and tissues is maintained in health, ammonia is nevertheless automatically furnished in conspicuous amounts when it becomes necessary to offset an impending acidosis. Hitherto the therapeutic procedure in such cases has been to furnish an alkali, commonly sodium bicarbonate, and thereby conserve the available supply of bases in the body from depletion. Recent studies have shown that a similar contribution of bases is afforded by a judicious selection of the dietary. There are foods which act as potential acids and others which function as bases in the organism. When burned up either in the laboratory or in the body-cells, they leave a preponderance of acid or base, as the case may be, in their ash. In this respect potatoes, apples, raisins, and cantaloupe, for example, are base-forming foods which depress the output of ammonia and increase the solubility of uric acid in the urine, as the experiments of Dr. Blatherwick in our laboratory have demonstrated; whereas meats, cereals and prunes (the latter with their content of benzoic acid) furnish acids in preponderance. Milk is intermediate in this respect. From the point of view here raised it is quite easy to accomplish as much with a liberal allowance of certain selected foods as with half an ounce of the familiar bicarbonate. To do this, however, implies that the physician must become as well acquainted with the properties of the foods as he appears to be with such novelties as amboceptors and complement, polyvalent vaccines and immune serums, salvarsan and epinephrin.

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Turning attention to another group, the fats, the question must have arisen in the minds of many a physician as to why certain representatives of these foodstuffs have so long maintained a reputation for superior merit in connection with nutrition. There are several fluid fats which are quite as available, or even more easily procurable than is cod-liver oil. Yet this product continues to enjoy a vogue that cannot be dismissed as the mere result of advertising propaganda. Cod-liver oil was a favorite, particularly in the dietotherapy of children, long before the present era of catch-phrase circulars and illuminated billboards. Its supposed unique virtues have called forth varied attempts at explanation. At one time it was the trace of iodin found in cod-liver oil which was believed to endow it with superiority over other fats like cottonseed oil and olive-oil having essentially the same physical properties and the same mixtures of glycerids, or fats proper. When this explanation no longer appeared tenable, the superior value of cod-liver oil was attributed to its ready digestibility. Lately Osborne and I have observed an unexpected efficiency in the product which is not shared by many other fats of similar superficial properties. We have fed growing animals (albino rats) on mixtures of isolated food substances including an abundance of fat in the form of commercial lard until, owing to the lack of some essential and as yet unknown ingredient of adequate dietaries, growth was checked. The replacement of a portion of the lard in the diet by cod-liver oil invariably brings prompt restoration of growth which continues uninterrupted until the adult stage is reached. Butter-fat and egg-yolk fat likewise contain a "growth-promoting"ingredient which appears to be more or less completely wanting in other dietary fats of both animal and vegetable origin. There is, for example, a marked difference between lard and the fat elaborated by the mammary gland. Whereas our experimental animals show symptoms of malnutrition with a cessa-

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tion of growth and decline in weight at the end of three months on a diet including lard as the sole fatty ingredient, when either butter-fat or cod-liver oil were used there was no sign whatever of failure after a year or more of uninterrupted feeding on the same diet day after day.

It is well known that all of the naturally occurring fats, such as have just been mentioned, are complex mixtures of true fats in the chemical sense, along with other substances including lipoids, pigments and compounds more soluble in fat than in the watery mediums of the body. The lipoids, in particular—cholesterol, lecithins, cephalins — have come into prominence in connection with various reactions involved in serum diagnosis and such phenomena as hemolysis, narcosis and absorption. Though long recognized as a constituent of the nervous tissues and certain pathologic products such as gall-stones, cholesterol has remained a compound of unknown function until very recent times. At the present moment the consensus of opinion appears to be that cholesterol is not ordinarily formed within the organism of the higher animals, and that these are dependent on the food intake to maintain the essential supply thereof. If this be true the ultimate source must reside in constructive plants; and assuming occasional losses from the body, doubtless with the feces, the store of cholesterol can be kept intact in man only by renewal from a suitable dietary. Ordinarily, in the adult living on a ration of products derived from many sources, no difficulty arises, because compounds of the cholesterol type are widely distributed and presumably found in all cells; but it is quite conceivable that in individuals like growing children or patients living on a diet restricted in character a real deficiency of cholesterol may ensue. It is known that the hemolytic phenomena of the red blood-cells are related to their lipoid content; and the therapy of the future may find a place for such substances as cholesterol, kephalin, etc., in the management of hemolytic

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anemias and other pathologic manifestations. Here, again, the intricacies of the diet cannot be overlooked by the intelligent physician of the future.

I have dwelt somewhat on the possibilities in respect to cholesterol and the growth-promoting components of milk-fat, egg-fat and cod-liver fat because it affords an opportunity to emphasize the hitherto largely unappreciated importance of factors in nutrition which cannot be expressed in terms of the familiar protein, fat and carbohydrate or in calories of energy. These factors may be inorganic in some cases, as is instanced by the reputed rôle of iodin and the wide distribution and alleged significance of manganese, boron, silicon and other elements in animal tissues; they are frequently organic in character. To class these varied factors together under the euphonius designation of "vitamins," as has lately been done, does not render less obscure the deficiencies which the absence of them from the diet may entail; nor does it give any clue to the mechanism whereby growth may be promoted, metabolism stimulated, or deficiency diseases engendered. Not only in those admitted instances of diet deficiencies such as beriberi and scorbutus, but also in numerous other clinical and experimental types of malnutrition there is need for further research. The proverbial adherence of the medical profession to dogmatic statement must be changed. Its members must become eager to take up the current views, study the underlying data as well as they can, and watch the consequences. Let us welcome the day when the food market and the kitchen are taken into consideration by the physician as seriously as are the apothecary's shop and the fashionable spas.

If in all the foregoing little mention has been made of the carbohydrates in nutrition, it has been because, in a general way, more has been known about them for a longer time than is true of the other nutrients. Even among the carbohydrates the principle of specificity comes into play. The physiologic carbohydrates

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are the six-carbon sugars, such as glucose, levulose and galactose. Unless an ingested carbohydrate can be converted into this simple type of sugar in the alimentary tract, its nutritive value is distinctly impaired or entirely lost. For this reason, all attempts to introduce more complex sugars, dextrins or starches directly into the blood-stream, for prompt distribution to the tissues which need them, have failed. In experiments conducted in our laboratory, animals have received subcutaneous or intraperitoneal injections of cane-sugar or milk-sugar for weeks and months without acquiring the capacity to utilize so little as a single gram introduced in this way in an undigested form. Carbohydrates like inulin and the galactan of agar-agar or of Irish moss can furnish no energy to the human organism because it is unable to digest them, that is, to convert them into simple sugars. The possibility of nourishing an individual in some way other than by the oral path evidently depends on introducing the nutrients in the form of those units—the amino-acids, simple sugars and fats—which normally constitute the actual pabulum of the hungry cells.

Physiology has borrowed its methods largely from the sciences of physics and chemistry. It also has a fruitful method of its own, that of experiments on animals and man. None of these procedures can be dispensed with. They corroborate and supplement each other, and they serve to test the hypotheses which give the impetus to progress. The medical profession must insist on conserving for the physiologic sciences the opportunity to continue its studies untrammeled by the passing prejudices of those who fail to appreciate its motives.

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INTERMEDIARY PROTEIN METABOLISM

OTTO FOLIN, PH.D.
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The subdivision of protein metabolism into intermediary metabolism and tissue metabolism indicates that we to-day are in possession of a much more detailed and accurate knowledge concerning animal metabolism than was available a few years ago. The controversy between Voit and Pflüger and their followers of the past generation as to whether the protein of the food must be incorporated in the protoplasm of the tissues before it is oxidized was an unprofitable and useless controversy, because it was too far in advance of the experimental knowledge of that period. We still do not know the spatial relationship between protoplasm and food materials at the moment of oxidation. On the other hand, we no longer speak of protein metabolism as being an oxidation process at all in the sense understood by Pflüger and Voit. While protein materials like other carbonaceous food materials are of course finally oxidized in the body, we now believe that the nitrogenous foodstuffs first undergo changes and chemical transformations involving the loss of all protein characteristics before they are completely destroyed by the oxidation processes involved in the production of heat and energy. Through the investigations of Kutscher (1898), and more recently of Abderhalden and his associates, it has been made clear that protein materials are completely decomposed into simple crystallizable products, the amino-acids, as a result of digestion. This discovery is fundamentally important for it has necessitated a complete revision of the earlier ideas and

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doctrines as to the nature of the metabolic processes involved in the utilization of protein as food. The old, burning question as to whether protein must be incorporated in the living tissue protoplasm before it is oxidized we now know was a false question, because the food protein has been altered beyond recognition even before it leaves the digestive tract and many of us no longer believe that protein as such is ever oxidized in the body.

The discovery that protein materials are split up into the simplest possible component parts during the digestion suggested two new fields for metabolism investigations. The first of these is the question as to whether simple amino-acids can be substituted for ordinary food protein, and which if any of the amino-acids are most indispensable for the maintenance of the animal organism. Much interest and important work has been done in this field, and it has become quite clear that some amino-acids are indispensable while others appear to be less necessary constituents of the food. The importance of all these data to the science of metabolism, if not to medicine, is self-evident in view of the fact that practically all the amino-acids can be manufactured. The second metabolism problem which required renewed investigations when the deep-seated character of protein digestion became clear concerns the subsequent history of the digestion products, and inasmuch as my own investigations lie in this field, I shall confine myself to a discussion of this problem.

Formerly it was generally believed that protein materials were but little altered by the digestion, and the protein of the food was supposed to pass into the blood-stream without any material alteration. Since no peptones were found in the blood the peptones formed during the digestion were supposed to be regenerated into more complex albuminous materials while passing through the mucous membrane of the

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intestinal wall just as fatty acids and glycerin are believed to be transformed into neutral fats during the absorption. This hypothesis seemed plausible, because it agreed with the experimental observations and because the animal organism was supposed to be dependent on the complex kind of proteins found in the blood. Many investigators, including Kutscher and Abderhalden, adhered to this explanation of what happens to the nitrogenous food in the intestinal wall even after it became certain that amino-acids and not peptones were the chief end-products of the digestion. Many physiologists, however, abandoned the protein regeneration hypothesis about ten years ago. To them it seemed highly improbable that the proteins should first be completely split up into amino-acids only to be immediately recombined into albumin and then again immediately broken down so as to give the urea, the elimination of which is greatly increased three or four hours after the intake of protein food.

In place of the protein regeneration hypothesis these dissenting physiologists assumed that the transformations of nitrogenous materials which take place in the intestinal wall are of a destructive rather than of a constructive character. Just as the digestive ferments convert the proteins into amino-acids within the digestive tract, so by a continuation of the digestive or hydrolytic processes most of the amino-acids immediately lose their nitrogen while passing through the intestinal wall and the liver. The prompt formation of urea, the supposedly large amount of ammonia in the portal blood, and many other features of the nitrogen metabolism were explained in a fairly plausible fashion by this hypothesis.

There remained, however, another possibility, namely, that the intestinal wall does nothing to the amino-acids but simply lets them through. From a theoretical aspect, this point of view has been the most plausible, and notwithstanding numerous early failures, the efforts to prove that the amino-acids are

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absorbed from the intestine without change have been continued, and now there is no longer any doubt about the fact that the end-products of protein digestion, the amino-acids, are quickly and easily absorbed. In fact, the speed of the absorption and the speed with which the amino-acids are distributed to all the different tissues in the body is one of the reasons why the earlier investigators failed to find the digestion products in the blood.

By means of the quantitative colorimetric methods devised in my laboratory, by means of Van Slyke's micro method, and by means of Abderhalden's qualitative ninhydrin test it is now easy to show that the amino-acids are absorbed. We have therefore now not the slightest reason for believing that the amino-acids formed during the digestion undergo any change while passing through the walls of the intestine.

The essentials of the story of protein digestion and absorption appear to be very clear indeed, and can be stated in two or three sentences. In the stomach the greater part of the protein is dissolved and much of it is converted into albumoses and peptones. In the intestine the dissolved products as well as the remaining undissolved residues are attacked by the trypsin and are thereby split up into amino-acids. The intestinal wall yields another ferment, erepsin, which, so to speak, prevents the absorption of albumoses and peptones by converting such as may escape the action of the trypsin into amino-acids — and the amino-acids as fast as they are formed are absorbed and transported by the blood to all parts of the body.

The absorption of amino-acids from the blood by the different tissues and organs is extraordinarily rapid, but is not complete. The communication between the blood and the tissues appears to be about equally good in either direction, and the accumulations of the nitrogenous digestion products in the blood and in the tissues reach therefore substantially the same level. This is, I think, true for the nitrogenous waste-

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products as well as for the digestion products, and if correct is important, for it means that by analyses of the blood we determine also the concentration or accumulation of the same products in the tissues.

The speed of the absorption is not the same for all tissues. The liver, for example, according to Van Slyke, absorbs amino-acids from the blood more rapidly than do the muscles even when the amino-acids are injected into the general circulation, and similar though smaller variations will doubtless be found in the speed of absorption revealed by different muscles; but such variations are probably only temporary, so that on the whole it will be found true, I think, that the concentration of a given nitrogenous product in the blood is the same as in such general tissues as the muscles.

In view of the established fact that the muscles absorb the greater part of the amino-acids coming from the digestive tract, it is no longer permissible to regard the liver as the chief seat of the urea formation. The greater part of the urea formation represents nothing but the removal of the nitrogen from amino-acids which are not needed for the rebuilding of new tissue material, and there is in my judgment no reason to doubt that the muscles which absorb the greater part of the amino-acids also form from them the greater part of the urea. Dr. Denis and myself have repeatedly emphasized this point of view, and recent studies made in the Harvard Medical School by Drs. Fiske and Sumner on the urea formation have clearly indicated that this process is not localized in the liver.

In its broad outlines the intermediary protein metabolism appears now comparatively simple and clear. The blood gets amino-acids from the digestive tract and all the tissues get them from the blood. From the amino-acid mixtures thus received each tissue rebuilds itself, and the amino-acids which are not needed for such synthesis are converted into urea and

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carbonaceous remainders. The latter are either converted into carbohydrates or are otherwise oxidized for the production of heat and energy. When protein materials from within the body are broken down, as in fasting and in fevers, these are in all probability digested in the tissues down to the stage of amino-acids, and from that stage on undergo the same transformations as do the amino-acids derived from the food.

According to this view it is to be noted that the specialization of functions which characterizes the higher animals does not extend very far into the field of general protein metabolism. All the tissues probably take part in this process roughly in proportion to the weight of living protoplasm contained in them. We cannot therefore expect to find the general protein metabolism materially or specifically altered because of this or that disease in any particular organ. I feel it incumbent on me to emphasize this aspect of the case, for we laboratory workers are often expected to elucidate diseases by metabolism studies when no such elucidation is possible on the basis of the available fund of knowledge of metabolism processes.

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THE SPECIFIC DYNAMIC ACTION OF THE FOODSTUFFS *

GRAHAM LUSK
NEW YORK

The conclusions to be reported in this paper are based on results obtained from 250 experiments on dogs. The animals rested quietly in a respiration calorimeter, at an environmental temperature of 26 C. (78.8 F.), and were subjected to different dietary conditions. Under these circumstances the simplest nutritional elements can be given and their effect on the heat production can be noted in hourly periods. This gives an idea of the interplay between the nutrient particles and the oxidizing tissues.

Dr. Du Bois will present the story from the point of view of investigations made with a similar though larger respiration calorimeter devised to determine the nutritional condition of the human being in disease.

All nutrient proteins, starches and compound sugars undergo fragmentation in the intestinal tract into simple chemical substances which are soluble in the fluids of the body. A polysaccharid like starch is broken up into glucose molecules; a disaccharid like cane-sugar (sucrose) is split into a molecule each of glucose and fructose, and lactose undergoes similar transformation into a molecule of glucose and one of galactose. Glucose, fructose and galactose are sugars which may be absorbed and carried to the living cells by the blood-current that bathes them. In similar fashion, the protein molecule is demolished in the intestine and broken into units such as glycocoll

* From the Physiological Laboratory of the Cornell University Medical College, New York.

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(amino-acetic acid), alanin (aminopropionic acid), glutamic acid, which is a dibasic amino-acid with five carbon atoms, and there are many others. These amino-acids are absorbed into the blood-stream and are carried to all the living cells of the body.

The result of the ingestion of food in any quantity is to increase the amount of heat production in the body. The extent to which the increase in metabolism occurs depends not only on the quantity, but also on the kind of food introduced into the blood-stream. Since the power to increase heat production exerted by protein, carbohydrate and fat is different for each substance, Rubner has defined this power as the specific dynamic action of the substance. The term is technical and cumbersome, yet the meaning is perfectly simple.

Every one has experienced the increased feeling of warmth after a hearty meal, and no one eats largely of meat in midsummer, for it increases heat production and consequently increases sweating.

The reason why the various foodstuffs increase heat production has not been entirely clear and it is this somewhat complicated problem with which the present paper deals.

In the first place, the increase in the heat production has nothing whatever to do with the increase in intestinal activity (*Darmarbeit*), a theory advocated by Zuntz; for the heat production is not increased by the ingestion of insoluble substances like bones or agar-agar, of saline cathartics even with the production of violent intestinal movements, or of Liebig's extract, which stimulates the glands to secretion. Likewise, incombustible substances such as urea and sodium chlorid when given in health, and glucose when given in diabetes, may be absorbed, carried in the blood-stream and eliminated in the urine without causing an increase in the oxidative processes. These facts have been established by Rubner, by Benedict and by others.

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When, therefore, the heat production of a dog rises 30 or 40 per cent. after glucose has been given, and even 90 per cent. after a large quantity of meat, it is evident that the cause of the specific heat production must lie, not within the absorptive or excretive mechanisms, but in the interplay between the living cells and the nutrient material, the sugars and amino-acids contained in the blood.

To determine the effect of sugars and amino-acids, experiments have been devised in which a dog was given a standard maintenance diet every evening, and eighteen hours thereafter, when the gastro-intestinal tract was free from food, the *basal metabolism* was measured. In Dog 3, on which eighty-two experiments have been performed, the basal metabolism was determined at different intervals and averaged 16.7 calories per hour. It scarcely varied from this during a period of nearly three months.

The effect of a substance to be tested was measured by giving it eighteen hours after the standard diet and noting the change which took place from the normal basal metabolism.

THE METABOLISM OF CARBOHYDRATES

After 50 gm. of glucose the heat production rose in one of the hourly periods to a maximum of 32 per cent. above the basal level. After 70 gm. of glucose a maximum rise was 38 per cent. With 50 gm. of fructose a similar rise was 44 per cent. and with 50 gm. of sucrose one of 48 per cent. Here then are very large increases. It has been shown on another occasion that 50 gm. of glucose are absorbed into the blood and disappear from the gastro-intestinal canal from three to four hours after its administration. At first the sugar-content of the blood increases, but then quickly falls to its normal percentage as the blood becomes more dilute, the latter fact being indicated by a fall in hemoglobin percentage. This greater volume of blood bears a greater number of readily

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diffusible molecules of sugar to the active tissues of the body. Following the end of the absorption of glucose, the kidney eliminates a large volume of the water which has caused the dilution of the blood, hemoglobin returns to its normal percentage, and the composition of the blood is again regulated by the liver and other organs through which it flows. In consequence of this, the heat production of the fourth or fifth hours after the ingestion of glucose regains the level of the basal metabolism. Those older experiments, which were performed during twenty-four-hour periods, do not show the very great temporary rise in metabolism after glucose ingestion, since calculations based on a long interval of time give no idea of the real state of affairs. Grafe's recent work on dogs which were overfed on carbohydrates throughout the day shows large increases in metabolism, as high as 33 per cent. above the level of an eighth day of fasting. This is fully explained in the light of the results given above.

But what is the cause of the increased heat production after the ingestion of sugar? On a former occasion I stated that it was due to the mass action of the sugar-molecules reacting with the living cells.

One might argue that this increased heat production is due to a breaking up of sugar in the inert fluids of the body and not in the cells themselves. An analogy is found in the living yeast-cell in which the greater part of the destruction of sugar is accomplished within the cell, itself, and a minor part in the surrounding fluid (Rubner). Recent work, however, has shown that the blood-plasma contains no glycolytic enzymes and cannot accomplish the breakdown of sugar.

Another explanation is offered by F. G. Benedict, who declares that acids formed in the intermediary metabolism of sugar are stimuli to increased metabolism. In substantiation of this, Benedict cites an instance of increased heat production following the

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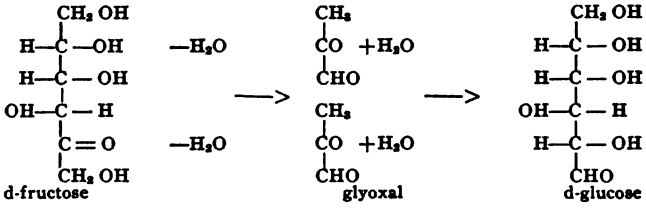
administration of 100 gm. of fructose to a diabetic patient, by whom this sugar was not oxidized, but in whom it was converted into glucose and eliminated in the urine. Benedict interprets this as due to the breaking up of fructose into acid and other radicals which are subsequently synthesized into glucose. Modern theory indicates that methylglyoxal is the principal intermediary substance in this transformation. Benedict's experiment was of such interest that it has been repeated, using phlorhizinized dogs, but it could not be confirmed. The diabetic animal did not oxidize the fructose; it also transformed fructose into glucose, but the metabolism was in no way increased.

The transformation of glucose into levulose may thus be written:



If this transformation takes place through methyl glyoxal, which is also believed to be an early metabolism product of glucose, then when a molecule of glucose breaks and forms two molecules of methyl glyoxal, these small three-carbon aldehyd radicals cannot, through their presence, place metabolism on a higher level. But through their mass action on the living cell, the oxidative processes involved in their destruction may cause metabolism to reach this higher level. This is the metabolism of plethora.

Experiments have also been made with alcohol alone and with alcohol and 50 gm. of glucose. When 9.4 gm. of alcohol were given alone, the heat production of the dog was increased 10 per cent. When alcohol was given with glucose the heat production was raised above the level to which glucose alone would have

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caused it to rise, and alcohol was oxidized in preference to carbohydrate as shown by a marked depression of the respiratory quotient. Here the alcohol, with its small molecule containing only two carbon atoms, proved to be the more available fuel.

METABOLISM OF AMINO-ACIDS

Dakin states that nine of the seventeen amino-acids which make up the composition of protein yield glucose when they are broken up in the body. Glycocoll with two carbon atoms and alanin with three are completely convertible into glucose, and the ammonia produced in this transformation forms ammonium carbonate in the blood, which, in turn, is converted into urea. These reactions are *endothermic,* that is to say, heat is absorbed in order to produce sugar and urea. In the transformation of 20 gm. of glycocoll into 16 gm. of glucose and 8 gm. of urea 17.1 calories are absorbed. When 20 gm. of alanin undergo this transformation, 3.6 calories are required. The amino-acid known as glutamic acid contains five carbon atoms, three of which are convertible into glucose. These three amino-acids have been extensively used in the experiments to be described, which were devised in order to interpret the cause of the very great increase in the heat production which follows the ingestion of meat.

A striking point is that if 20 gm. of glutamic acid be given to a dog there is no increase whatever in the heat production of the animal. Twenty gm. of glutamic acid yield 13 gm. of sugar which, of itself, is too small to increase the production of heat. This amino-acid which is convertible into sugar and urea may be given without appreciable change in the heat production. If, however, 20 gm. of glycocoll be given, a very great increase in heat production follows. The higher metabolism lasts for seven hours. Twenty gm. of glycocoll containing 40 available calories increases

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the heat production by 30 calories during this period. This is greater than the 17 calories absorbed in the intermediary production of sugar and urea. Ten gm. of glycocoll produce just half the effect which 20 gm. accomplish.

Twenty gm. of alanin, containing 73 available calories, increases the production of heat by 20 calories, and 30 gm. of alanin cause an increase of 30 calories. The result which follows administration of the amino-acids is like that which occurs after meat ingestion, in that the amount of the increase is proportional to the quantity of material metabolized.

What is the cause of these great increases in heat production? Twenty gm. of glucose may have no effect whatever on the heat production, and 70 gm. may cause an increase of 20 calories, yet 20 gm. of glycocoll, which is convertible into only 16 gm. of glucose, raise the heat production by 30 calories, a height that 70 gm. of glucose cannot bring about.

What is the reason?

At the International Congress of Hygiene and Demography, held in Washington, I presented the view that it was due to the stimulation of the protoplasm to higher activity through the mass action of accumulated amino-acids. Folin had shown that amino-acids were quickly absorbed and were retained by the tissues, and he believed that their metabolism was of slow development, none taking place in the first hour. Experiments which have since been instituted have led me to believe that the highest heat production is coincident with the highest metabolism of amino-acids and that the amino-acids themselves do not lie in the tissues without being involved in a highly active metabolism. A year ago, Miss Wishart performed experiments on dogs killed in the height of excessive protein digestion, and failed to find any great increase in the quantity of non-protein nitrogen in the muscles. Another convincing reason of the

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rapidity of the metabolism of amino-acids, such as glycocoll and alanin, lies in the speed with which Csonka found that the sugar derived from them is eliminated in phlorhizin glycosuria. If 20 gm. of glycocoll be administered to a diabetic dog, 16 per cent. of the sugar derivable from it is eliminated in the urine of the first hour. At the end of five hours, 86 per cent. of the sugar which could be derived from glycocoll metabolism has been eliminated. The elimination of the sugar is almost as rapid as when a comparable quantity of glucose is given to the dog. The point to be emphasized is that this method of determining the rapidity of the metabolism of glycocoll and alanin shows that the early hours of their highest metabolism is coincident with the maximum increase in the production of heat within the dog after their ingestion.

After all, might not the great rise in metabolism be due to the oxidation of glycocoll or alanin as so much refuse which cannot be used as fuel for the cells? To determine this, glycocoll and alanin were given to the dog in the diabetic condition and the metabolism was measured. The heat production rose in both instances. Since the amino-acids were not oxidized but were eliminated as sugar and urea in the urine, the increased heat production must have been due either to the stimulus exerted by them on the living protoplasm or to the stimulation produced by the oxy- or keto-acids formed from them during their transformation into sugar.

That specific intermediary acids formed in metabolism are the real stimuli is indicated by the fact, first shown by Rubner, that when ingested protein is reconstructed into body protein the amino-acids involved in this reaction do not cause an increase in the heat production.

It does not seem probable, however, that this stimulation is induced by all acids, for the strong dibasic glutamic acid is without effect. It may be that lactic

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acid derived from alanin and subsequently converted into glucose is an effective stimulus, whereas methyl glyoxal, which is a problematic cleavage product of glutamic acid and is convertible into sugar, may not be an effective stimulus to higher oxidation.

In phosphorus poisoning, which is accompanied by formation of lactic acid, the total heat production is above the normal, although a high protein metabolism may here also be an accessory factor. In high altitudes, where lactic acid is always produced, metabolism increases.

The rise in heat production in diabetes may be partly induced by the great increase of fat in the blood and by the increased protein metabolism and not entirely by the diabetic acidosis as suggested by Benedict. For, in the dog, diabetic acidosis plays a very subsidiary part and yet an increase in the metabolism of the fasting phlorhizinized animal of 75 per cent. above the level of the basal metabolism has been noted.

Even though the cause of the increased heat production after protein ingestion be due largely to a stimulation caused by the presence of certain oxy- or keto-acids, yet the metabolism induced thereby can be defined as the metabolism due to amino-acid stimulation, to contrast it with the metabolism due to plethora in the presence of food molecules in abundance.

In conclusion, it may be stated that living cells metabolize carbohydrates and fats in increased quantity when these are present in large amounts in the surrounding fluid, and that they are also stimulated to a higher heat production during the metabolism of certain amino-acids to an extent which is entirely out of proportion to the energy value of those amino-acids, and which may indeed be independent of their energy value.

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THE TOTAL ENERGY REQUIREMENT IN DISEASE

AS DETERMINED BY CALORIMETRIC OBSERVATIONS*

EUGENE F. DU BOIS, M.D.
NEW YORK

The total energy requirement of an individual is represented by his heat production or, in other words, his total metabolism. If food of a total caloric value equaling the heat production be taken, the normal man will remain in nutritive balance. As a rule, the appetite in health regulates the intake to a nicety, although some of us need the guiding influence of the bathroom scales and the restraining influence of tight clothes. In disease, on the other hand, the appetite is no index of the requirement and the nutrition of the cells is at the mercy of the physician. Likewise, the stomach and kidneys are at his mercy, and he must regulate the diet so that it will nourish the cells without throwing too much work on damaged organs or oxidative functions of the body. It is often just as important to know the proper dosage of foods as to know the proper dosage of drugs.

The most satisfactory instrument for measuring the heat production is the respiration calorimeter[1] of the type devised by Atwater and Rosa in Middletown, Connecticut. Such a calorimeter, modified for clinical purposes, has been constructed by the Russell Sage Institute of Pathology in the wards of the Second Medical Division of Bellevue Hospital. Since its com-

* From the Russell Sage Institute of Pathology in affiliation with the Second Medical Division of Bellevue Hospital.

1. Atwater and Rosa: Description of a New Respiration Calorimeter, 1899, Bull. 63, U. S. Dept. of Agr. Benedict and Carpenter: Respiration Calorimeter for Studying the Respiratory Exchange and Energy Transformations of Man. Carnegie Instit. Wash., 1910, Pub. 123.

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pletion a year and a half ago, it has been used in 142 observations. A number of normal controls have been studied, but most of the subjects have been patients from the adjoining metabolism ward in which all diets are weighed and analyzed. This ward is under the control of the institute and those in charge of the calorimeter.

On the morning of the observation a patient, usually without his breakfast, is placed within the calorimeter where he remains for three or four hours. He finds himself on a comfortable bed in a well-ventilated box about the size of the lower berth of a sleeping-car. He can amuse himself by looking out of the windows, but since the scenery is monotonous he usually dozes for about half the time and allows his mind to vegetate for the other half. Absolute rest is essential, and the only movements permitted are those connected with turning in bed from time to time. All such movements are registered automatically by a device which is delicate enough to record even the small amount of work done by a man in stroking his mustache.

As the subject lies in the calorimeter the oxidation of the various foodstuffs within his tissues produces a certain amount of heat each hour. Approximately three-fourths of this heat leaves the surface of the body by means of radiation and conduction. This heat is removed from the calorimeter by a system of cooling pipes and is accurately measured by electrical thermometers. About one-fourth of the heat is removed from the body by the vaporization of water from skin and lungs. This water vapor is caught and measured in a sulphuric acid bottle.

If the body temperature should rise, it is obvious that some of the heat produced would be stored in the body itself and not eliminated. The estimation of this heat in short periods is a matter of great technical difficulty since the changes in rectal temperature, which are recorded every four minutes by means of a very accurate electrical thermometer, may

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not represent the average temperature changes of the body. Other thermometers strapped in the axillae and on the surface of the thorax or abdomen demonstrate that the different parts of the body show temperature curves which are by no means parallel, and that one part of the body may even be cooling while another part is warming. If the body temperature of a man of average size rises 0.5 degree centigrade it means that from 25 to 30 calories have been stored in the body in addition to the calories eliminated from the body. Such large changes, amounting to one-fourth or one-third of the total heat production for an hour, have been observed within an hour, and it is obvious that any inaccuracy in the determination of the average body temperature causes an error in the method of direct calorimetry. While the error may be large in a single hourly period, it becomes very small in an observation lasting three or four hours and seldom exceeds 5 per cent.

The heat production is measured not only by this method of direct calorimetry but also by the more accurate method of indirect calorimetry. The box is ventilated by a rotary fan which sucks the air out of one end of the chamber, drives it through a bottle of sulphuric acid to remove all moisture, then through a bottle of soda lime to remove the carbon dioxid and finally returns the same air, absolutely pure and odorless, to the other end of the calorimeter. As the patient uses up oxygen, this gas is supplied automatically from a small tank. By weighing these various bottles and tanks at the beginning and end of each hour it is a simple matter to determine the carbon dioxid produced and the oxygen consumed. Knowing the relative proportions of these and also the nitrogen of the urine one is able to calculate with great accuracy the grams of protein, fat and carbohydrate oxidized by the patient during that hour. Knowing the heat produced by the oxidation of 1 gm. of each of these substances it becomes a simple matter to calculate the total heat production.

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The two methods of direct and indirect calorimetry agree within 5 per cent. in about three-fourths of the experiments, and in many cases the figures are almost identical as is shown in Chart 1, that of one of the normal controls. In the total of all the experiments so far calculated, involving the measurement of 23,000 calories, the two methods agree within 1.6 per cent.

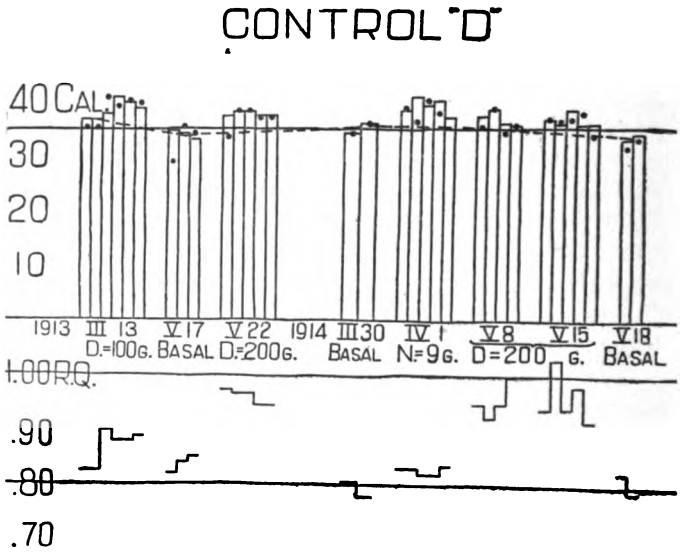

Chart 1.—Control D. Showing the basal metabolism in hourly periods and the effects of food in increasing the heat production. Each column represents the calories per square meter of body surface as determined by the method of indirect calorimetry. The round dot represents the calories as measured by the method of direct calorimetry. *D* stands for glucose; *N*, nitrogen in the form of casein and egg albumin. These were taken approximately one hour before start of observation except on March 13, 1913, when the glucose was taken after the basal metabolism had been determined in two one-hour periods.

As yet no disease has been found in our somewhat limited series of observations in which there is any consistent disagreement between the two methods. This is a matter of considerable import, since the previous work in the study of the heat production in disease has been done almost exclusively by the method of indirect calorimetry. The agreement of the two methods shows that the calculations by indirect calor-

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imetry have not been vitiated by any profound and unsuspected changes in the intermediary metabolism.

It is comparatively easy to determine the total metabolism but difficult to find the exact normal figure with which to compare the results. We have measured the basal heat-production of a number of normal persons of varying weights and have grouped them with the normal controls examined by Benedict in his bed calorimeter, all determinations being made on individuals at complete rest, from fourteen to sixteen hours after the last meal. It will be seen in Chart 2 that if we express the metabolism in terms of calories per square meters of the body surface per hour, the results will group themselves close to the average figure of 34.2 irrespective of the body-weight. The dotted line represents the average of forty-nine normal controls studied by various types of apparatus in many laboratories. It is evident that the level is the same for persons whose weights are between 40 and 50 kg. (from 88 to 110 pounds) as for those whose weights are between 60 and 70 kg. (from 132 to 154 pounds). On the other hand, if we express the results in terms of calories per kilogram per day we have a line of averages which falls steadily as the weight increases. In order to have a single base-line which need not be changed for persons of varying weights we therefore compare all our results with the average normal figure of 34.2 calories per square meter per hour.

While the metabolism of a given individual remains fairly constant for years, there may be a variation among normal men of 10 per cent. above or 10 per cent. below the average. Further study will perhaps show even a greater variation. The metabolism is increased in childhood, diminished in old age, and probably depends, to a great extent, on the nutrition of the body and the mass of active protoplasm. Nevertheless, Rubner's law that the metabolism of different individuals and different species of animals is proportional to surface area does hold good within cer-

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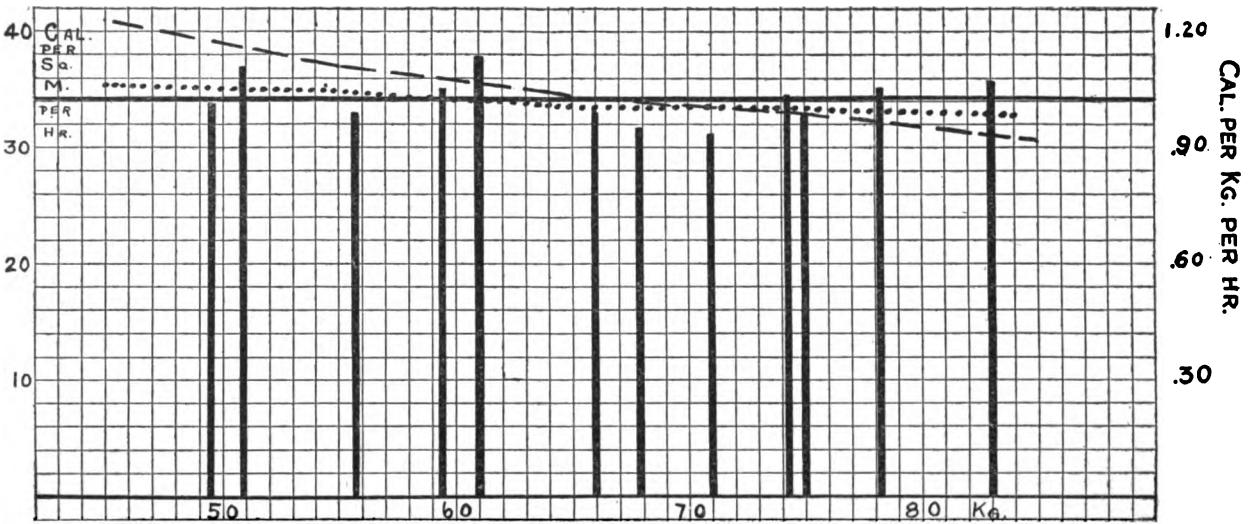

Chart 2.—Basal metabolism of normal men from fourteen to sixteen hours after the last meal. Columns represent calories per square meter per hour produced by subjects in bed calorimeter. Horizontal line drawn at 34.2, the average. Interrupted lines represent averages of 49 normal controls studied with various types of respiration apparatus, arranged in five groups according to weight. Dotted line shows the results expressed according to surface area. Dash line represents the same 49 controls with the results expressed in terms of calories per kilogram per day.

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tain limits. These limits may be wide when we try to compare the metabolism of babies, guinea-pigs, athletes and fat men, but, as we have shown above, the limits are narrow for a group of men of approximately normal build.

Voit and Rubner demonstrated the fact that the taking of food raised the heat production, and the term of specific dynamic action was applied to this phenomenon. The cause of this was little understood until Lusk[2] studied the matter in great detail, making hourly determination of the heat production of dogs in the small Cornell calorimeter. His work on dogs has been duplicated on normal men as nearly as the human stomach will permit, but, of necessity, the amounts of food are relatively smaller and the increases in heat production distinctly less marked. Chart 1 shows the basal metabolism of control D and the effects of glucose and protein meals. The increase after glucose amounts to from 8 to 10 per cent. and after protein to 12 per cent.

Since the time of Voit and Pettenkofer a great deal of work has been done on the subject of the respiratory metabolism and total heat production in disease. Magnus-Levy[3] established the fact that in exophthalmic goiter the energy requirement was greatly increased even to a point as high as from 50 to 70 per cent. above the normal and showed that in cretinism and myxedema the heat production might be as low as from 45 to 50 per cent. below the normal. Kraus,[4] Grafe,[5] Rolly[6] and others demonstrated that in typhoid fever the metabolism was raised to from 30 to 40 per

2. Lusk: Animal Calorimetry, Papers II, III, V, VI, Jour. Biol. Chem., 1912, xii, 349; xiii, 27, 155, 185; Cause of the Specific Dynamic Action of Protein, Arch. Int. Med., December, 1913, p. 485; The Specific Dynamic Action of the Foodstuffs, this issue, p. 824.

3. Magnus-Levy: Gaswechsel bei Thyroiden, Berl. klin. Wchnschr., 1895, xxxii, 650; Ueber Myxödema, Ztschr. f. klin. Med., 1904, lii, 201.

4. Kraus, Fr.: Ueber den respiratorischen Gasaustausch im Fieber, Ztschr. f. klin. Med., 1890, xvi, 160.

5. Grafe: Untersuchungen über den Stoff- und Kraftwechsel im Fieber, Deutsch. Arch. f. klin. Med., 1911, ci, 209.

6. Rolly: Experimentelle Untersuchungen über den Stoffwechsel im Fieber und in der Rekonvalescenz, Deutsch. Arch. f. klin. Med., 1911, ciii, 93.

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cent. above the normal. Grafe[7] has recently shown that the heat production is low in certain stuporous conditions and increased in lymphatic leukemia and cancer. The total heat production in diabetes has been studied by many observers with varying results, but Benedict and Joslin,[8] using the calorimeters in Boston, have shown that in cases of severe diabetes with acidosis there is an increase in metabolism.

The study of the metabolism in typhoid fever and the effects of the high-calory diet was begun in Bellevue Hospital in 1911 in association with Dr. Warren Coleman. The small Benedict apparatus was used and the calculations were made according to the method of indirect calorimetry. A summary of the results will be presented by Dr. Coleman[9] in another Section but it may be stated that the heat production of the patients was not raised by the administration of large amounts of food during the height of the fever. The matter seemed so important that it demanded investigation by the use of the calorimeter.

Observations were therefore made on nine typhoid patients during the course of the fever and convalescence. The first part of the results on one patient, who was in the calorimeter twenty-two times, is shown in Chart 3. This patient enjoyed the work with the calorimeter. His original infection was by no means mild and he had one relapse which was severe and a second relapse which scarcely interrupted a rapid convalescence. Throughout the disease he was so well nourished that his weight remained practically constant.

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7. Grafe: Beiträge zur Kenntniss der Stoffwechselverlangsamung bei stuprösen Zuständen, Deutsch. Arch. f. klin. Med., 1911, cii, 15; Die Steigerung des Stoffwechsels bei chronische Leukaemie und ihre Ursachen, Deutsch. Arch. f. klin. Med., 1911, cii, 406; Das Verhalten von Gesamtstoffwechsel und Eiweissumsatz bei Karzinomatösen, Deutsch. med. Wchnschr., 1914, xl, 518.

8. Benedict and Joslin: Metabolism in Diabetes Mellitus, Carnegie Inst. Wash., 1910, Pub. 136; A Study of Metabolism in Severe Diabetes, 1912, ibid, Pub. No. 176.

9. Coleman, W.: The Effects of Food on Metabolism in Typhoid Fever, read in the Section on Pharmacology and Therapeutics at the Sixty-Fifth Annual Session of the American Medical Association, Atlantic City, N. J., June, 1914.

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It will be noticed that during the fever his total metabolism was from 40 to 50 per cent. above the average normal and that it was scarcely increased at all on the days when the observations were made shortly after the ingestion of 100 gm. of glucose or 64 gm. of protein. A study of the nitrogen in the urine in this and three other cases shows that the percentage of calories derived from protein during fever when the patients were fasting was seldom raised

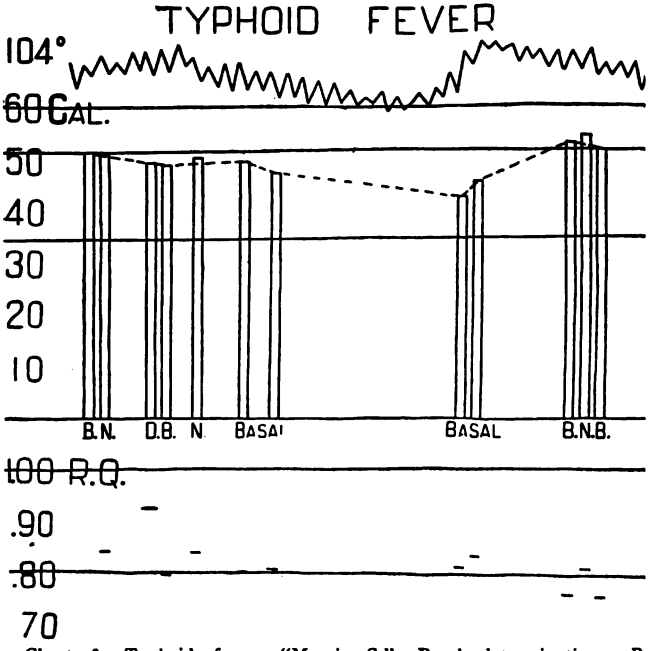

Chart 3.—Typhoid fever, "Morris S." Basal determinations, *B* connected by dash line. *N*, protein meal. *D*, from 100 to 200 gm. of glucose. Results expressed in averages.

above the upper normal limit of 20 per cent., although one patient early in his disease derived 28 per cent. of the calories from protein.

As the temperature falls the total metabolism diminishes and the percentage of calories derived from protein drops to 10 or 15 per cent. During convalescence heat production may be from 20 to 30 per cent.

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above normal, and at this stage glucose and protein exert as great a specific dynamic action as in health.

The diseases of the thyroid gland have been studied with particular interest since the level of the total metabolism seems to be the best index of the activity of the thyroid secretion. In one cretin who was 36 years old but had the mental and physical development of a child of 8, the total energy requirement was from 18 to 25 per cent. above the normal but was raised almost to the normal on the third day of treatment

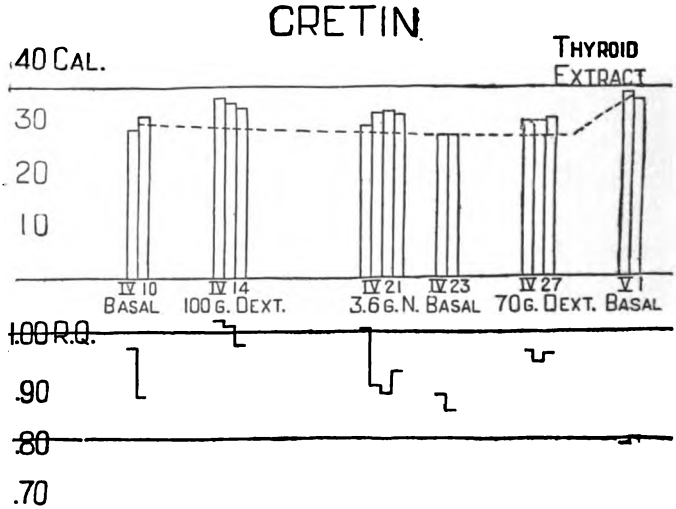

Chart 4.—Cretinism results expressed in hourly periods. Thyroid-extract treatment begun two days before last observation.

with thyroid extract (Chart 4). The specific dynamic action of food was normal in this patient.

A series of seven patients with exophthalmic goiter were studied before and after treatment in an effort to guide the therapeusis in this disease, which is remarkable for the number of "remedies" that do not give relief. Luckily we were able to show some beneficial effects of treatment, although good results were obtained by rest and careful feeding alone. One patient, who had shown moderately severe symptoms

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for one year, was studied over a considerable period of time. If we look at the results as expressed in Chart 5, we notice that, in marked contrast to the cretin, the metabolism is 80 per cent. above the normal, falling to 60 per cent. on rest and good food. Von Noorden[10] has stated that the specific dynamic action of food and especially of meat is increased in fever and in exophthalmic goiter. We have seen that this

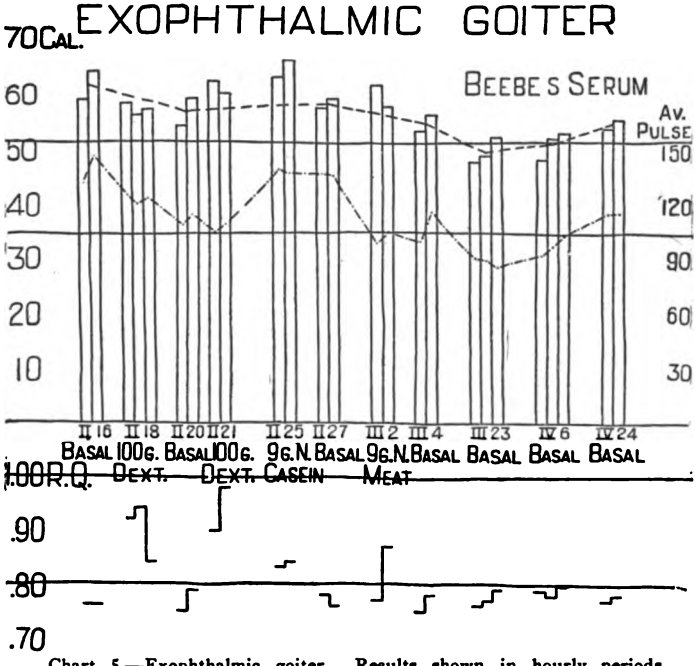

Chart 5.—Exophthalmic goiter. Results shown in hourly periods. Dash line connects basal determinations. Dot-dash line represents average pulse during the period of observation in calorimeter.

was not the case in fever and it was evidently not true of this patient, since his metabolism was not raised even so much as that of a normal man by glucose, casein and meat. After treatment by Dr. S. P. Beebe with his antithyroid serum, there was a distinct improvement in the man's condition and fall in the

10. Von Noorden: New Aspects of Diabetes, New York, E. B. Treat & Co., 1912, p. 20.

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metabolism. After the first month there was a rise in metabolism to its former level but the general improvement continued perhaps, however, at a slower rate.

A young woman who was up and about, though not quite able to continue her work as trained nurse, showed the extraordinarily high metabolism of 92 per cent. above the normal. After ligation of the arteries by Dr. John Rogers, the metabolism fell to 60 per cent. above the normal and the patient's condition was distinctly improved.

One young woman with a mild case of the disease showed a marked rise in metabolism four days after ligation of the four thyroid arteries, indicating that the operative procedure had temporarily increased the activity of the gland. Later there was distinct improvement in her general condition and a fall in the heat production to its former level.

Other conditions have been studied in a few cases and while the results need confirmation, they are suggestive and may lead to a better understanding of the various factors which influence the heat production in disease. One patient with splenic anemia, whose hemoglobin was 25 per cent., had a metabolism 11 per cent. above the normal; another with pernicious anemia for which a splenectomy had been performed, showed an increased metabolism amounting to 20 and 26 per cent. above the normal, while his hemoglobin was only 20 per cent. This confirms the older work, which demonstrated that there is no diminution of oxidation as a result of the low percentage of hemoglobin. In a patient with nephritis a change in blood-pressure from 195 to 160 caused absolutely no change in heat production. One man with acromegaly had a metabolism 18 per cent. above the normal, another with symptoms of deficiency of the secretion of the posterior lobe of the hypophysis gave results sometimes high and sometimes normal but he was so stupid that he made a very unsatisfactory subject. Another man

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with malaria was studied at the height of his fever, and also six days later when his attacks were cured. With a temperature of 104 F. the metabolism was 83 per cent. above the average, with a normal temperature of 21 per cent. A patient with auricular fibrillation, mitral stenosis and greatly enlarged heart but without orthopnea, was observed three times lying flat on his back and twice propped up with a back-rest. We were astonished to find the metabolism which was normal when he was propped up was 8.5 per cent. higher when he was lying down and were even more surprised to observe the same phenomenon in the only normal control we studied in this posture.

The increases due to fever, hyperthyroidism and the like, are probably small compared to the increase caused by muscular work in delirium tremens or convulsions. Even slight restlessness or mild excitement causes a rise in heat production which is distinctly appreciable in the calorimeter.

In conclusion, it may be well to emphasize the fact that the nutrition of a patient depends absolutely on the relationship between his energy production and his food-supply. If the organism fails to obtain food from without it will draw on its own glycogen stores, fat depots and supplies of body protein.

129 East Seventy-Sixth Street.

ABSTRACT OF DISCUSSION

ON PAPERS OF MENDEL, FOLIN, LUSK AND DU BOIS

Dr. S. J. Meltzer, New York: For more than thirty years I have been moving on the periphery of laboratory work on one side and clinical medicine on the other side. Well do I remember the wide gulf which separated the laboratory men from the men in clinical medicine. To-day we have heard four men telling of their work in the sciences of medicine and telling it to a large audience, consisting, as far as I can judge, chiefly of men who are active in the practice of medicine. There are reasons for more congratulations. We heard to-day four men telling of their own original work in the scientific part of medicine. In the times gone by the laboratory man was satisfied to discuss work which was done on the other side of the Atlantic; to-day the scientific men in Europe take

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profound notice of the work done in this country. Again, in the past, laboratory men used to be proud of the fact that they had nothing to do with the practice of medicine. To-day the laboratory men are anxious to do work in fields which are of special interest to the practitioner. Mendel speaks of "gaps and guesses" in scientific reports. I am certain that a thousand years from now "gaps and guesses" will have a prominent place in scientific reports. The gaps which we fill out by our investigations, we do by means of guesses, and they reveal to us new gaps. Liebig's theories are mere guesses for us to-day, to be forgotten. But they were instrumental in filling up many gaps by facts. Theories are of value because they help us to discover facts, and as long as they can do that they are still of value to us. I wish to say to Dr. Mendel that the amboceptor theory of Ehrlich, of which he spoke somewhat disparagingly, has brought out thousands of important facts in immunity, and is still capable of bringing out new facts. I am still more sorry that Dr. Mendel spoke in a somewhat disparaging manner about salvarsan. The synthetic creation of such a specific substance like salvarsan is one of the most wonderful discoveries made in recent times in the sciences and practice of medicine. The discovery by Dr. Mendel in conjunction with Osborne, that butter does contribute to the growth of rats, while lard does not do it, is perhaps the best he has ever made. This discovery was made by the empirical method; they do not tell us on what hypothesis they found it; neither what is the nature of the "vitamin" which differentiates lard from butter. We call this method, when used in science, the inductive method. But it differs from the empirical method which the physician uses merely by the application of criticism. Criticism is here necessary to differentiate the coincidental from the causal connection. Here the physician must learn from the scientist. A patient may get better merely while he is taking a drug and not because he takes it. But there is one point which the scientist has to learn from the physician; it is the principle of *non nocere;* be sure that you do not do any harm. For instance, the knowledge that the minimum diet is of no perceptible immediate harm to animals is no evidence that it will be of no harm, acute or chronic, to human beings. Neither do we know that infants will behave like rats regarding the difference between butter and lard.

Dr. F. M. Pottenger, Los Angeles, Cal: Does this difference in the absorption of fats apply to all animals alike, whether herbivorous or carnivorous and also to man?

Dr. E. P. Joslin, Boston, Mass.: I should like to ask Dr. Lusk if he has an explanation to offer as to why the metabolism is not increased by food in typhoid fever?

Dr. Allan Eustis, New Orleans: I wish to discuss that part of the subject in which Dr. Mendel refers to the amins

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formed by the putrefaction of the several amino-acids in the intestinal tract. This has a broad practical application in clinical medicine, as each of these amins has its own specific physiologic action, just as the amins from the vegetable kingdom, represented by the alkaloids strychnin, morphin, etc. Tyramin (para-oxyphenylethylamin), which is yielded by tyrosin on putrefaction, is capable of raising the blood-pressure in a dog from 110 to 250 mm., while in man it has produced a rise to 180 mm. I have made application of this fact in treating nearly five hundred cases of arterial hypertension, in which the systolic blood-pressure has been kept down to 140 or 150 mm. by dietetic means alone. It must be remembered that caseinogen of milk yields 7 per cent. of tyrosin on digestion which contra-indicates milk as an ideal food in this condition. Inasmuch as all animal proteins yield large amounts of tyrosin they are also contra-indicated, while the best results will be obtained from a cereal and fruit diet. The clinical application of the formation of histamin (beta-imidazolylethylamin) from histidin can be made in the treatment of bronchial asthma and urticaria. When injected intravenously, this amin causes violent bronchial spasm similar to an attack of asthma, while when rubbed vigorously into the skin a solution of 1:1,000 causes a typical urticarial lesion. Along dietetic lines alone, I have been able to give relief in 168 cases of bronchial asthma and twenty-six cases of urticaria, some of the asthmatics having had the disease for twenty years. A diet for these conditions should be based on the fact that the yield of histidin in the more common types of proteins is: hemoglobin, 10 per cent.; sturin, 12 per cent.; caseinogen, 2.5 per cent.; 2.5 per cent., and gliadin, 1.4 per cent. Articles of diet which yield little or no hisidin may be allowed. It must not be concluded that all cases of arterial hypertension are due to the absorption of tyramin from the intestinal canal, nor that all cases of asthma and urticaria are due to histamin absorption; but it must be admitted that these amins have a decided practical bearing in clinical medicine and must be reckoned with.

Dr. Douglas Head, Minneapolis: I should like to ask whether any observations have been made as to the effect of butter feeding on heat production in long, thin-chested, enteroptotic persons as compared with those of short, stocky build?

Dr. A. J. Carlson, Chicago: I think you all know that in this particular field America has made a distinct contribution to science. I have been impressed by the interest taken in this symposium by the members of the American Medical Association. If there is anything new of importance in the practice of medicine it is this modern work in nutrition. And it is evident that when the laboratory men really have something to say the clinical men will listen.

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Dr. Victor C. Vaughan, Ann Arbor, Mich.: Will Dr. Folin explain one point on which he did not touch? We recognize that in enterodigestion the proteins are split into amino acids. Dr. Folin holds that the amino acids are absorbed directly into the walls of the intestines, a view held by many others. The proteins are carried to each cell and each cell utilizes what is suited to its needs. This I dare say is all right. One point which he should explain, is important. There must be some synthesis of amino acids in the body. The sero-albumin and globulin in the blood are specific proteins, each coming from different sources. These are taken into the blood, and by the brilliant work of Folin and others we are now able to demonstrate the presence of amino acids in the body. Now there must be some synthesis somewhere. We must not lose sight of the fact that enterodigestion is not the only form of digestion that occurs in the animal body. There is in the blood a digestive process as much as in the intestines. A proteolytic ferment is present in the blood, a trypsin and an antitrypsin, and the understanding and the adjustment of the relation of these two factors is one of the great problems of physiologic chemistry.

Dr. Otto Folin, Boston, Mass.: Undoubtedly all will agree that the question asked by Dr. Vaughan is too much for even a laboratory worker to attempt to answer. The question that Dr. Vaughan has really asked is "How are tissues formed?" I have discussed the subject of the destruction and the history of protein in the body from the point of view of food and from that of destructive metabolism. The matter has two aspects, a destructive and a constructive; the constructive or the synthesis of protein is synonymous with the formation of tissue itself and is far from the transformation of amino acids into urea. There are two distinct aspects of metabolism. I considered only the destructive processes, the breaking down, and these only as produced by the class of agents sometimes known as enzymes. Frequently enzymes are of the destructive variety. They are hydrolytic and there is a degradation form of reaction. As to the difference between the enzymes and the proteins and the subject of hydrolytic and synthetic ones, all can discuss this part of the subject as well as I can.

Dr. Graham Lusk, New York: Replying to Dr. Joslin's question, "Why is there no increased metabolism in typhoid fever after giving an excess of food?" I would call attention to the fact that under normal conditions the basal metabolism is measured by the heat production of a subject whose gastro-intestinal tract is free from food and who is living at an environmental temperature of 26 C. (78.8 F.). If, however, the temperature be lowered there is an increase in the heat production although, under these circumstances, the

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ingestion of food may now produce no specific dynamic action. Furthermore, it is known that, if the metabolism be increased to a certain point through carbohydrate, the addition of amino acids to the diet may cause no further increase in metabolism. Likewise, it should be remembered that in fever the protein metabolism can never be reduced to the low level present in the normal person, so that when amino-acids enter the organism under these circumstances they tend merely to replace those already breaking down in large quantity. It is therefore apparent why in fever the metabolism may have already been lifted to such a level that ingestion of food causes no further increase.

Dr. Eugene F. Du Bois, New York: A comparison of the metabolism of enteroptotic persons with that of short, stocky men would be interesting, but we have had no opportunity of making such observations. From a theoretical consideration it would seem that the enteroptotic, undernourished persons would have a rather low metabolism, and it would seem that muscular persons would have the higher metabolism. Yet from clinical observations it appears that the enteroptotic persons have the greater energy requirement. We have at times tried to guess the actual heat production of individuals and groups of patients but have found that sometimes we were as much as 25 per cent. from the true figures as determined by the calorimeter.

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